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ANTIBIOTIC SUBSTANCES FROM BASIDIOMYCETES V. PORIA CORTICOLA, PORIA TENUIS AND AN UNIDENTIFIED BASIDIOMYCETE[*]

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It was noted in a previous report from this laboratory[1] that *Poria corticola* (71280) and *Poria tenuis* (67942) showed considerable antibacterial activity. Each of these fungi was found to produce two antibiotic substances which are closely related chemically. A third unidentified fungus, B841, isolated from White Cedar and obtained through the courtesy of Dr. Dow V. Baxter, University of Michigan; produced the same two antibiotic substances. We have named these substances nemotin and nemotinic acid, respectively.

Preliminary Observations.—Each fungus was grown in Petri dishes on corn-steep, thiamine-peptone and Difco potato-dextrose agars. When coated with an AC medium and tested by the streak method,[1,2] none of the three fungi affected the growth of *Escherichia coli;* each inhibited the growth of *Staphylococcus aureus* (H). B841 showed marked antibacterial activity against *Mycobacterium smegma*. The activity was most pronounced when the fungus was grown on corn-steep agar. *P. corticola* and *P. tenuis* did not inhibit *M. smegma* when tested by the streak method.

Each fungus grown on the three media was active against *Staph. aureus* as tested by the agar disc method.[1,2] The size and character of the inhibition zones were determined by the age of the colony, the position of the disc in relation to the fungus colony, and the medium on which the fungus was grown. Discs cut from colonies of *P. tenuis* and *P. corticola* produced characteristic "bull's eye" zones of inhibition.[3] The zones produced by *P. corticola* were fainter than those observed with *P. tenuis*. Discs of B841 usually produced a clear inhibition area around the disc

surrounded by a small ring of partial inhibition. Inhibition zones 30 mm.
in diameter were obtained from discs cut from 6-day old colonies of this
fungus.

When tested by the agar disc method against *M. smegma*, B841 showed
marked activity; inhibition zones with diameters of 20–30 mm. were
obtained. The other two fungi were nearly or completely inactive. This
is of special interest because the culture liquids of these two fungi were
found to contain material active against *M. smegma*, as is pointed out later.

Liquid Cultures.—Each of the fungi was grown in still culture at 25°C.
in 2800-ml. Fernbach flasks with beech shavings plus one liter of corn-
steep medium. *P. corticola* grew the most rapidly but produced liquid
with the least antibacterial activity. Although the activity of culture
liquids of *P. tenuis* became as great as those of B841, it usually took longer.
The mats of *P. tenuis* tended also to become thicker than those of B841
and· were consequently less suitable for reflooding. For these reasons
most attention was devoted to fungus B841.

The activity of culture liquids of B841 varied from 64 to 256 dilution
units per milliliter against *Staph. aureus* after about four weeks of incuba-
tion, at which time the mycelium covered the entire surface of the liquid.
Liquids from mats reflooded with fresh sterile corn-steep medium averaged
128 dilution units per ml. in from 3 to 5 days after reflooding. It was
possible to reflood the mats several times until the thickness of the mycelial
mat made this technique inconvenient. The original corn-steep medium
had a pH of about 5.5. The pools of culture liquids ranged from pH 4.1
to pH 4.9. Old mats produced sufficient HCN to retard the growth of
susceptible organisms grown in the same incubator.[4] An incubation
temperature of 25°C. was more satisfactory than 20° or 30°C.

Cultures of B841 in the corn-steep liquid medium were shaken at room
temperature on a Gump rotary shaking machine at 200 rotations per
minute on a circle 2¼ inches in diameter. After 2 to 3 weeks, the liquid
assayed 128–256 dilution units per ml. against *Staph. aureus*. Tests were
negative for HCN in the shake cultures. Substitution of sucrose, lactose,
levulose, glycerin, maltose, mannitol, soluble starch or galactose for
dextrose in the corn-steep medium in shake cultures gave no better or
poorer ·results. Liquids with good activity were obtained in a potato-
dextrose broth.

Separation and Concentration of Antibacterial Substances.—Two frac-
tions, one neutral and the other acidic, were prepared from culture liquids
of each of the three fungi. The acid fraction contained an antibacterial
substance, nemotinic acid; the neutral fraction a closely related material,
nemotin. No evidence for the presence of other antibacterial substances
was obtained.

The general procedure[8] in preparing the two fractions was as follows.

The filtered culture liquid amounting to several liters was acidified to pH 3, extracted with $1/_{10}$ volume of methyl isobutyl ketone, the ketone evaporated *in vacuo* nearly to dryness, alcohol added and the evaporation repeated. After several evaporations with alcohol, most of the ketone had been removed. The heating of the still was done carefully to avoid heating of the flask above the liquid line with consequent decompositon of the antibiotic substances. The residue in the still was dissolved in a small amount of alcohol and about 50 ml. ether added. A brown precipitate formed in the ether solution prepared from 67942 and B841. The brown precipitate was inactive and was discarded. The ether solution was extracted with a small amount of pH 5.8 to 6.0 M/2 phosphate buffer. This removed much of the pigment. The total amount of material removed was small but it contained a major fraction of the colored substances. The ether solution was then extracted several times with $1/_{10}$ volume of 2 per cent sodium bicarbonate solution to remove the acidic substances. The bicarbonate solutions were combined, acidified and extracted with ether. This acidic fraction contained nemotinic acid. The ether solution after the bicarbonate extraction contained the very weakly acidic and neutral substances including nemotin.

Properties of the Acidic Fraction (Nemotinic Acid).—The free acid was soluble in ether, chloroform, alcohol, methyl isobutyl ketone and acetone and slightly soluble in water and hexane. Solubility in water was about 16 μg. per ml. On drying in air at room temperature a brown, chloroform insoluble, inactive varnish was formed. However, nearly all the ether could be removed by careful evaporation without formation of insoluble material or loss of antibacterial activity. The brown insoluble product was formed in aqueous or ether solution on standing at 11°C. and higher temperatures. The greater the concentration, the sooner the insoluble material formed. Titration in 20 per cent alcohol indicated a monobasic acid with a $p_{k_a} = 4.95$ and equivalent weight of 205. The optical rotation was $[\alpha]_D^{25} = +270$ (0.44 per cent in ethanol).

Only minor differences were observed in the absorption spectrum and antibacterial activity of nemotinic acid after standing at room temperature for one hour at pH 3.6, 6.15 or 9.4. At pH 12.6 substantial changes were noted.

Properties of Neutral Fraction (Nemotin).—Nemotin was soluble in ether, chloroform, methyl isobutyl ketone and acetone. It was less soluble than nemotinic acid in water and hexane. Nemotin formed a brown insoluble product on drying. It was not extracted from ether by 0.1 N NaOH. The optical rotation obtained from nemotin was $[\alpha]_D^{23} = +202$ (1.2 per cent in ethanol).

Nemotin was found to change in solutions of pH 6.0 and higher into

another neutral substance with quite different antibacterial properties and absorption spectrum (Fig. 1). At the dilutions used (10 μg. per ml. or less) the conversion was monomolecular. This conversion product we have named nemotin A. The higher the pH of the solution, the more rapid the conversion. The rate of change at pH 6.0 and 25°C. was sufficiently slow to permit measurement of the absorption spectrum. Transformation was one-half completed in 3 hours at 37°C. in pH 7.2 buffer. A measurable amount of nemotin was not transformed by the bicarbonate
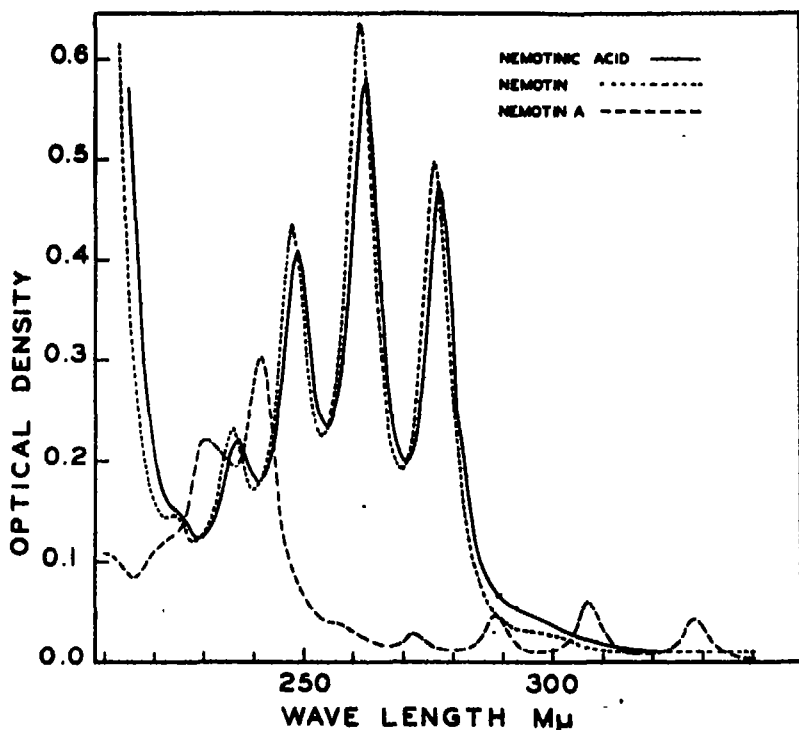
FIGURE 1

Absorption spectra of nemotinic acid, nemotin and nemotin A in pH 6.0 buffer. Concentration of 10 μg. per ml. for nemotin and nemotinic acid and of 1 μg. per ml. for nemotin A.

extraction of ether in removing acids. At high pH, nemotin A underwent further changes which resulted in loss of antibacterial activity and decrease in ultra-violet absorption.

Absorption Spectra.—The absorption spectra of preparations of nemotinic acid, nemotin and nemotin A were measured with a Beckman DU spectrophotometer. The solutions were made in one-tenth molar pH 6.0

phosphate buffer. The results (Fig. 1) are reported as optical densities obtained in a 1-cm. cuvette for a concentration of 10 μg. per ml. for nemotin and nemotinic acid, and 1 μg. per ml. for nemotin A. · The solutions of nemotin A were measured at concentrations between 0.6 and 10 μg. per ml., depending upon the absorption. The preparations of nemotin and nemotinic acid used were obtained from *P. corticola* and seemed to be of fair spectroscopic purity.

The absorption spectrum obtained with nemotinic acid was characterized by high, sharp peaks at 237, 249, 263 and 277.5 mμ. That for nemotin was almost identical; the peaks occurred at 1 to 2 mμ shorter wave-lengths. The differences between the spectra of nemotinic acid and nemotin may have been caused by impurities.

The absorption spectrum of nemotin A had peaks at 231, 242, 272, 289, 307 and 328 mμ. The high peak of nemotin A at 242 mμ corresponds to a valley in the absorption spectrum of nemotinic acid. By the transformation of nemotin to nemotin A at suitable pH and temperature, the presence of 2 per cent of nemotin as a contaminant of nemotinic acid could be detected.

Identity of the Antibiotic Substances Formed by the Three Fungi.—Preparations of nemotinic acid from culture liquids of each of the three fungi had nearly identical absorption spectra and the same relative antibacterial and antifungal activities within the limits of error inherent in the methods. The same comments can be made for nemotin. Nemotin prepared from culture liquids of each of the three fungi changed to nemotin A at the same rate at pH 7.2 and 37°C. It is probable that the three fungi form the same two antibiotic substances.

Antibacterial Activity.—The antibacterial activities of nemotinic acid, nemotin and nemotin A were determined by the serial dilution methods in use in this laboratory (6). The antibacterial activities of nemotin and nemotinic acid prepared by a counter-current distribution method[7] and by the procedure given in this paper were the same within the limits set by the precision of the measurement of antibacterial activities. The activity in the following table is expressed as the minimum inhibitory concentration in μg. per ml. (p = partial inhibition):

BACTERIUM	NEMOTINIC ACID			NEMOTIN			NEMOTIN
	71280	67943	B841	71280	67943	B841	A, B841
Bacillus mycoides	64	250	250	16	64, 32p	32	32
Bacillus subtilis	0.25	0.25	0.25	0.125	0.0625	0.0625	4
Escherichia coli	64	125	125	16	64	32	250
Klebsiella pneumoniae	64	250	250	32	32	32	250
Mycobacterium smegma	8	4	4	2, 1p	2, 1p	1	32
Pseudomonas aeruginosa	64	125	250	125	250	250	250
Staphylococcus aureus	1	1	1	2	2	2	2

Through the courtesy of Dr. Walsh McDermott and Dr. W. C. Robbins of the Cornell Medical College, the activity of several preparations was tested against *Mycobacterium tuberculosis* (H37RV strain) in a Dubos-oleic-acid-albumin medium with and without Tween 80. After one week, 7 μg. per ml. of nemotinic acid completely inhibited the growth of this organism.

The antibacterial substances retained their activity after incubation at 37°C. for 3 hours in the presence of 5 per cent human blood in beef extract medium with 0.7 per cent NaCl.

Antifungal Activity.—The fungistatic activity of the two substances prepared from organism B841 was measured by serial dilution methods in a peptone medium at pH 6. The dilution tubes were inoculated with a spore suspension of the fungus concerned. *Trichophyton* was incubated at 30°C., the others at 25°C. The activity reported in the following table was the minimum inhibitory concentration in μg. per ml. (*p* = partial inhibition):

ORGANISM	NEMOTINIC ACID	NEMOTIN
Aspergillus niger	125	4
Chaetomium globosum (USDA 1042.4)	>250	4
Gliomastix convoluta (PQMD 4c)	>250	16, 8*p*
Memnoniella echinata (PQMD 1c)	125*p*, 250*p*	0.5
Myrothecium verrucaria (USDA 1334.2)	250	4
Penicillium notatum (832)	32, 16*p*	0.03
Phycomyces Blakesleeanus (+ strain)	>250	4, 2*p*
Saccharomyces cerevisiae (188)	16	0.06
Stemphylium consortiale (PQMD 41b)	>250	16, 8*p* 64, 32*p*
Trichophyton mentagrophytes	64	4

Animal Toxicity.—The toxicity of nemotinic acid prepared from *P. tenuis* was determined by injecting 0.5 ml. of the neutralized acid made in 0.9 per cent sodium chloride into a tail vein of 16 g. Carworth Farms CFI white male mice. There were five mice in each treatment group.

All mice which received 5 mg. died within 6 hours. Four of the mice given 3 mg. died within 6 hours. Three of the mice receiving 2 mg. died within 24 hours. None of the mice which were given 1 mg. died within 10 days. The substance was acutely toxic with an LD_{50} of about 2 mg. per mouse or 125 mg./kg. (1 mg. = 1000 dilution units against *Staph. aureus*).

One of us became sensitive to these antibiotic substances with symptoms similar to but milder than those produced by contact with biformin.[3]

[1] Robbins, W. J., Hervey, A., Davidson, R. W., Ma, R., and Robbins, W. C., *Bull. Torrey Bot. Club*, **72**, 165–190 (1945).

[1] Harvey, A., *Ibid.*, 74, 476–503 (1947).

[2] Robbins, W. J., Kavanagh, F., and Hervey, A., *Ibid.*, 75, 502–511 (1948).

[3] Robbins, W. J., Rolnick, A., and Kavanagh, F., *Mycologia*, in press.

[4] An 8-tube Craig counter-current distribution system using ether and pH 6.33 M/2 phosphate buffer was found to separate nemotinic acid from nemotin.

[5] Kavanagh, F., *Bull. Torrey Bot. Club*, 74, 303–320 (1947).

[6] Anchel, M., Polatnick, J., and Kavanagh, F., *Arch. Biochem.* in press.

[7] Robbins, W. J., Kavanagh, F., and Hervey, A., these PROCEEDINGS, 33, 176–182 (1947).

FACTORS DETERMINING SOLUBILITY AMONG NON-ELECTROLYTES*

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This address has been prepared with the purpose of giving to non-specialists, so far as possible within the allotted time of fifteen minutes, a large-scale survey of the present status of the theory of solubility of non-electrolytes, with emphasis upon the methods used in attacking the various phases of the problem. That the problem is indeed a complex one is well illustrated by a system of seven liquid phases, a photograph of which I recently published.[1] Its components are heptane, aniline, water, "per-fluorokerosene" (approximately $C_{12}F_{24}$), phosphorus, gallium and mercury. These molecular species differ so strongly among themselves as to resist more or less completely the mixing effect of thermal agitation. The differences are in part qualitative, and include metallic character, dipole moment and hydrogen bridging, but in part, also, quantitative, differences in the strength of the "van der Waals" or, more appropriately designated, "London" forces. Professor F. London explained these forces as the consequence of quantum mechanical interaction between the molecular electron clouds. The strength of the attraction depends upon the number and what we may crudely call the "looseness" of the electrons. It is expressed in terms of polarizability, α, and "zero point energy," $h\nu_0$, the energy of electrons in their ground states, an energy which persists even at absolute zero. The expression for the potential energy between molecules of two species, 1 and 2, at distance, r, is

$$\epsilon = \frac{3\alpha_1\alpha_2}{2r^6} \cdot \frac{h\nu_{0,1} \cdot h\nu_{0,2}}{h\nu_{0,1} + h\nu_{0,2}}. \tag{1}$$

Higher terms for second order effects have been added as a result of more refined analysis but these are hardly significant for our purpose, particularly

because there is evidence[a] that the molecular fields of *polyatomic* molecules are not best described as radial from their geometrical centers but as extending, rather, from peripheral atoms or "orbitals." We find, fortunately, that such uncertainties are at least partly cancelled by the process, presently to be described, upon which our general theory is based. There are, however, three corollaries of the theory which are to be noted as most significant to our purposes: (1) the attraction is very short in range, (2) the attractive potential between pairs of unlike molecules is simply related to that between pairs of the like molecules and (3) these interactions, unlike those we call "chemical," do not saturate each other, and therefore the potential energy of a mass of liquid may be expressed as an integral of all the pair potentials.

The process we have adopted to serve as the basis for our theory of solubility is the mixing of two pure liquid components by isothermal distillation to form a solution in which their mole fractions are x_1 and x_2, respectively. The present exposition will, however, appear simpler if we think of distilling one mole of either component (let us select no. 2) into a very large amount of solution in which its mole fraction is x_2. The change in free energy accompanying such a transfer is

$$\bar{F}_2 - F_2^0 = RT \ln (f_2/f_2^0) = RT \ln a_2, \tag{2}$$

where f_2 denotes its fugacity (a corrected vapor pressure) in the solution and f_2^0 its fugacity in its pure liquid. We shall henceforth use activity, defined as $a_2 = f_2/f_2^0$. When one forms a saturated solution, he applies the "solute" at an activity which he can control, by pressure, in case of a gas or vapor, or calculate, in case of a solid, from its melting point and heat of fusion.

Now the change in free energy in this process may be regarded as the resultant of the accompanying changes in heat constant, $\bar{H}_2 - H_2^0$ and in entropy, $\bar{S}_2 - S_2^0$, as given by the pure thermodynamic equation,

$$\bar{F}_2 - F_2^0 = \bar{H}_2 - H_2^0 - T(\bar{S}_2 - S_2^0). \tag{3}$$

Ideal Solutions.—Let us begin with an ideal case, which, like all ideals, can be only rarely approached and never quite realized, *i.e.*, a solution of two molecular species having equal intermolecular attractions and equal molal volumes. The energy and heat of transferring any molecule from its own pure liquid into the solution is then zero, hence

$$\bar{F}_2 - F_2^0 = -T(\bar{S}_2 - S_2^0). \tag{4_1}$$

Now the change in entropy accompanying our process is a logarithmic function of the ratio of the respective "probabilities" of finding a molecule of species 2 in its pure liquid and in its solution. (I need not take time to explain why it is logarithmic, because many in my audience already know

and the others can easily find out if they wish.) In our ideal solution, since the molecules are of equal size, and since thermal agitation keeps them mixed with maximum randomness, the ratio of probabilities is $1/x_2$ and this, translated into entropy, gives

$$\bar{s}_2 - s_2{}^0 = -R \ln x_2. \tag{4_2}$$

Substituting this and equation 2 into 3, gives $RT \ln a_2 = RT \ln x_2$ or $a_2 = x_2$, which is Raoult's law; a relation used in all modern texts on physical chemistry to derive the various ideal solution laws.

Regular Solutions.—It has been our good fortune to find that thermal agitation usually suffices to give practically complete randomness of mixing, with two non-polar species, in spite of even large differences in their molecular forces, and, therefore, if their molal volumes are not significantly different, the entropy of transfer can still be close to the ideal entropy, $-R \ln x_2$. This uniformity regarding entropy causes a regularity in solution behavior evident in the family of solubility curves illustrated in figure 3, which suggested the term, "regular solutions." But now, in order to have a solubility equation, we must express $\bar{H}_2 - H_2{}^0$ in terms of
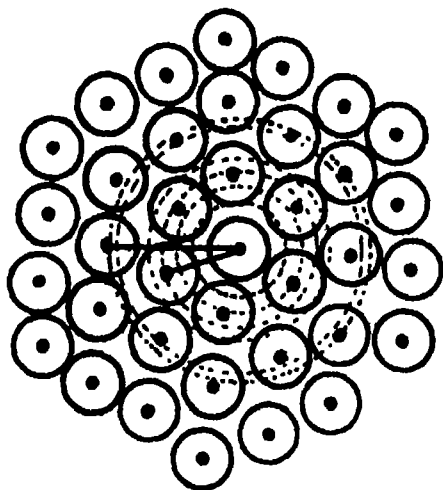
FIGURE 1

Cross-section of structure of an ideal liquid.

the pure components. If we were dealing with pure and mixed crystal lattices of equal, known lattice dimensions, we could add all the pair potentials to give the lattice energy. We can apply equivalent reasoning to liquids, but, instead of a summation over all the discrete distances in a crystal lattice, we must integrate over a continuous "distribution function" which expresses time-average frequency for all pair distances in the highly blurred, short range order in the liquid. The meaning of this function, $\rho(r)$, is easily grasped by referring to figure 1, which represents a cross-section of the instantaneous arrangement of molecules around a central one. The volume of a spherical shell of thickness dr of large radius is simply $4\pi r^2 \, dr$, and since the density of molecules in the liquid is the Avogadro number divided by the molal volume, N/v, the number of molecular centers in the large shell is $(4\pi Nr^2 \, dr)/v$. But when r is small, the presence of the central molecule makes the probability of molecular

centers in such a shell much higher than unity at about 2r, moderately higher at about 4r, etc. This varying probability is the distribution function, $\rho(r)$. Its form is nearly the same for all equally expanded liquids if plotted as $\rho(r/r_{max})$ as illustrated for four liquid metals in figure 2. By combining $\rho(r)$ with the pair potential function, $\epsilon(r)$, we can obtain an expression for the potential energy of a mole of liquid,

$$\mathbf{E} = \frac{2\pi N^2}{\mathbf{v}} \int \epsilon(r)\rho(r)r^2\, dr. \tag{5}$$

We may extend this treatment to the potential energy of a solution, where the (random) molecular distribution involves the relative molal
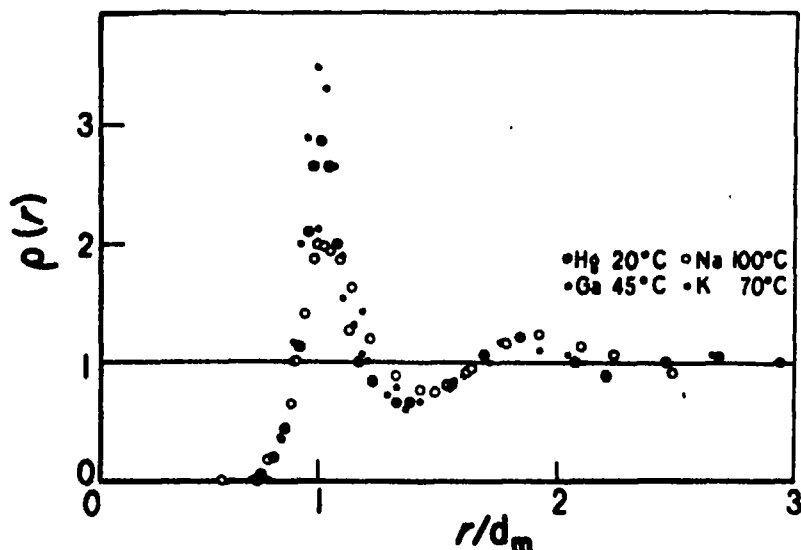
FIGURE 2

Distribution functions, $\rho(r)$, for liquid mercury, gallium, sodium and potassium plotted against r + molecular diameter.

volumes, and the total potential energy involves the pair potentials, ϵ_{11}, ϵ_{22}, ϵ_{12}, and the last can be eliminated by assuming the geometric mean. The model is simple but the mathematical steps in arriving at the final equation are far beyond the scope of this presentation, so I give only the result,

$$\mathbf{E}_2 - \mathbf{E}_2{}^0 = \mathbf{v}_2\phi_1{}^2(\delta_2 - \delta_1)^2, \tag{6}$$

where ϕ_1 denotes volume fraction of that component and $\delta = (\Delta \mathbf{E}^v/\mathbf{v})^{1/2}$, the square root of the energy of vaporization per cc., an easily obtainable property of both pure liquids. With this expression for the heat effects, our solubility equation becomes

$$RT \ln a_2 = v_2\phi_1{}^2(\delta_2 - \delta_1)^2 + RT \ln x_2. \qquad (7)$$

The δ-values play so predominant a rôle in determining solubility relations that we have designated them as "solubility parameters" and find it useful to have at hand tables of their numerical values at the standard temperature, 25°. Illustrative values for common substances are given in Table 1.

Solubility Relations of Iodine.—The high attractive field of iodine molecules, indicated by its solubility parameter, 14.1, is correlated with the great spread in the solubility curves plotted in figure 3, and the enormous deviations from ideal behavior in those solvents low in the plot. These solutions present a particularly exacting test of the theory underlying equation 7. Iodine solutions offer another important advantage in that one can easily distinguish two classes of solutions—one, the violet solutions, whose color, identical with iodine vapor, shows that chemical interaction is absent, and that they should behave "regularly"; the other class, yellow

TABLE 1

SOLUBILITY PARAMETERS OF IODINE, δ_2, CALCULATED FROM SOLUBILITY IN VARIOUS SOLVENTS AT 25°

SOLVENT	SOLUBILITY, 100 x_1	δ_1	δ_2 (CALC.)
n-C$_4$F$_{16}$	0.0182	5.7	14.2
SiCl$_4$	0.499	7.6	13.9
CCl$_4$	1.147	8.6	14.2
TiCl$_4$	2.15	9.0	14.1
CS$_2$	5.58	10 0	14.2
1,2-C$_2$H$_4$Br$_2$	7.82	10.4	14.1
Ideal	25.8		

to brown in color, indicating specific, "chemical" interactions. I invite your attention to the following features of the solid curves, all of which refer to violet solutions.

First, the parallelism in the slopes of these curves was what originally suggested applying to such solution the term "regular," and the regularity is obviously a matter of entropy, because it has to do with the temperature coefficient of a free energy relation.

Second, the positions of the curves accord remarkably well with the demands of equation 7, as seen by the small variations in the values of δ_2 for iodine calculated from the δ_1 values and the experimental solubilities, illustrated in Table 1 for several representative solvents. Reversing the procedure, calculating x_2 using $\delta_2 = 14.1$ for all, would obviously not lead to serious error. Particularly striking is the agreement in the case of fluoroheptane. When I first heard of the remarkable extremes in the solvent powers of fluorocarbons, I wondered whether they would overstrain the theory. The point for iodine in perfluoro normal heptane at 25° was the first to be determined, and when it was found to fit equation 7

so well I felt confident that all other solubility relations of fluorocarbons would fit the theory, and this expectation has since been abundantly confirmed.

Third, a particularly striking confirmation was furnished by the pre-
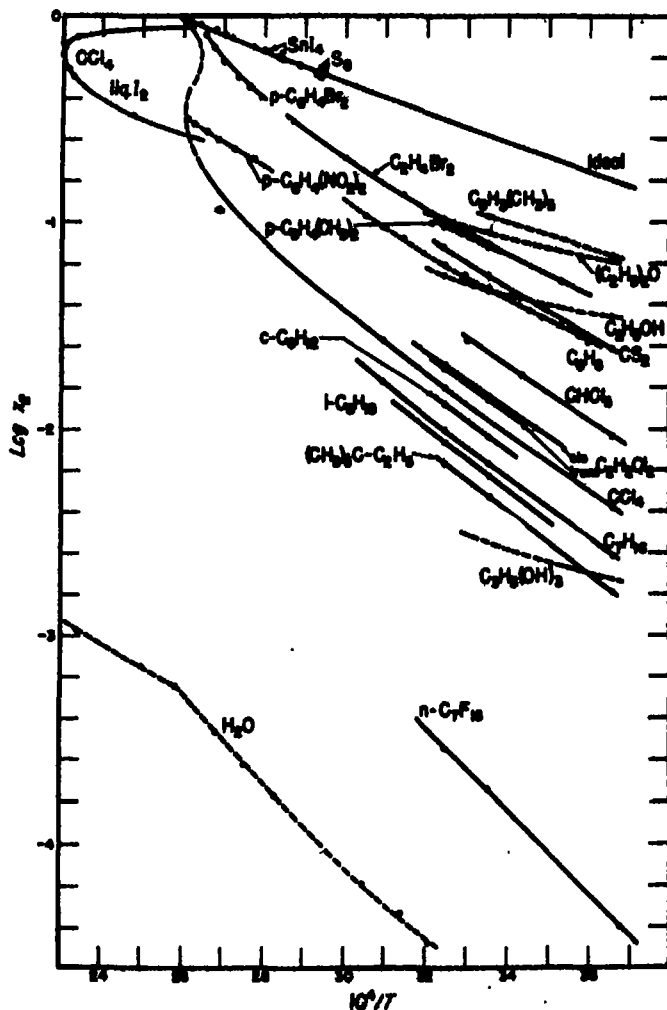
FIGURE 3

Solubility curves for iodine.

diction of the liquid-liquid loop for iodine and carbon tetrachloride, seen in figure 3, and this was later found close to the position predicted.

Fourth, moderate dipole moments, other than the very exposed ones leading to hydrogen bridging, may affect the δ-value slightly while not

interfering with regularity, as seen by the curve for chloroform, and the practical identity of the curves for *cis-* and *trans-*dichloroethylenes, whose dipole moments are 1.89 and 0.00 Debye units, respectively.

Solvated Solutions.—In cases where the unlike molecules react chemically, the 1-2 attraction is enhanced, and is no longer equal to the geometric mean of the 1-1 and the 2-2 attractions; the heat absorbed on dilution will be reduced over its regular solution value, and its sign may even be reversed; and the disorder and hence the entropy of the process will be less than ideal. But all these effects are more or less specific, not to be calculated by any general theory. The solutions of iodine in benzene, toluene, xylenes and mesitylene present illustrations of extraordinary interest.

Iodine dissolves in them to give solutions with brown colors increasing in the above order. They show strong absorption bands in the ultraviolet[3] whose intensities, when iodine and an aromatic are dissolved together in a "violet" solvent in varying concentrations, indicate a 1:1 solvate. We explain the interaction as that between an acid and a base; the aromatics are bases or "electron donors," whose strengths increase in the above order, and the iodine, an acid, or "electron-acceptor." The equilibrium constant for the reaction $I_2 + C_6H_6 = I \cdot C_6H_6$ in carbon tetrachloride was found to be 1.72, and the constant for the corresponding reaction with mesitylene is 7.2. (Anyone who thinks that none but "proton-acceptors" can act as bases will not be able to understand this.)

The solubility curves for these aromatics would all lie close to the curve for chloroform if there were no solvation; the curves are lifted to the positions seen in the figure[4] by the solvation, and the amount of displacement agrees remarkably well with that calculated by aid of the equilibrium constants for the acid-base interactions. It should be emphasized that the solvation is related to this displacement and not to departure from the ideal solubility curve. The common practice of interpreting inconstant partition coefficients in terms of chemical equilibria may be quite unrealistic and lead to conclusions very different from those which could be drawn from light absorption.

The Entropy of Mixing Molecules of Different Size.—It will be recalled that in deriving the term $-R \ln x_2$ for the entropy of transfer from pure liquid to solution, equality of molal volumes was not taken too seriously, however, the necessity for this limitation was not appreciated until recently because solutions are known which approximate closely to Raoult's law despite some inequality in molal volumes, but there have recently been discovered solutions of high polymers which depart strongly from Raoult's law while showing little or no heat of mixing, making it clear that it is entropy which must be held responsible. This led to efforts on the part of several investigators to formulate the entropy of mixing monomer molecules with small integral multiple polymers, but Flory and Huggins,[5]

simultaneously and independently, succeeded in deriving an expression for the entropy of solution of flexible, chain molecules occupying multiple sites in a quasi lattice with solvent molecules occupying single lattice sites. The same formula can be derived without the limitations of either linear polymers or lattice structure. The method used can be illustrated by the problem of properly expressing the disorder presented by blocks scattered randomly over the floor of a room, a picture with which, as a father and grandfather, I have become very familiar. It is obvious that the degree of disorder is some function of the number of blocks and also of the area of the floor, but it depends also upon the size of the blocks, since, in order to make the process of creating order truly analogous to parallel task with molecules which we cannot see, we must locate our blocks by wandering over the floor blindfold and barefoot. We will be able, of course, to locate blocks of larger area more easily than smaller ones. The entropy of transfer from pure liquid to solution formulated by aid of analogous probabilities for molecules, led, much to my satisfaction, to the same expression as the one obtained by Flory and Huggins. I here write it in the form
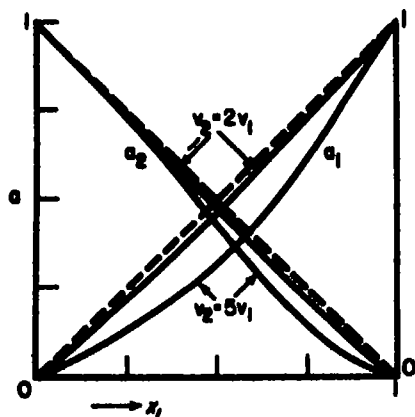
FIGURE 4

Effect upon activity of disparity in molal volumes for $v_2/v_1 = 2$ and 5.

$$\bar{s}_2 - s_2{}^0 = -R \ln \phi_2 + \phi_1\left(1 - \frac{v_2}{v_1}\right). \qquad (8)$$

When $v_1 = v_2$, the right-hand member reduces to $-R \ln x_2$, as it should if correct.

The order of magnitude of this correction is shown in figure 4 for two different ratios of v_2/v_1, 2 and 5. One sees that the departure from ideal entropy is rather small for the ratio 2, but very considerable for the ratio 5. Most pairs of ordinary organic solvents have molal volume ratios of 2 or less, but if one wishes to deal with solutions in fluorocarbons of small molecules such as nitrogen, or chlorine, the Flory-Huggins expression for entropy becomes significant. Since both heat and entropy may be non-ideal, it is useful to substitute the expression in equation 8 for $-R \ln x_2$ in equation 7, giving

$$RT \ln a_2 = v_2 \phi_1 (\delta_2 - \delta_1)^2 + RT \left[\ln \phi_2 + \phi_1 \left(1 - \frac{v_2}{v_1} \right) \right]. \quad (9)$$

Recent measurements of the solubility of nitrogen[6] and of chlorine in fluorocarbons can be better correlated by means of equation 9 than by equation 7, since the volume ratios, v_1/v_2, in these solutions run as high as 5, and the solubilities are markedly increased thereby.

The remarkably low solubility parameters for the fluorocarbons are mainly the result of their large molecular volumes. Although a pair of fluorocarbon molecules would attract each other more strongly than a pair of their hydrocarbon equivalents equally separated, the latter pair approach each other so much more closely at their equilibrium distance that, in view of the inverse sixth power of attractive potential, a cubic centimeter of liquid hydrocarbon not only contains many more molecules than the same volume of a corresponding fluorocarbon but they attract each other much more strongly.

[*] AUTHOR'S NOTE: The variation of this contribution from the coldly impersonal style customary in these PROCEEDINGS is the result of a remark by Professor P. Debye that it "should be published just as it was delivered." I trust that readers will feel that its purpose has been served thereby.

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NOTE ON A RELATION IN DIRAC'S THEORY OF THE ELECTRON

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A relation in Dirac's theory of the electron will be described here which allows one to show that the hyperfine structure energy of an electron in a Coulomb field is proportional to $<r^{-3}>$, where the $< >$ denote the expectation value of the enclosed operator. This is an extension of the well-known relation of proportionality in non-relativistic theory of the hyperfine energy to $L(L + 1)<r^{-3}>$ and hence to $a_H<r^{-3}>$, where L is the azimuthal quantum number and a_H is the Bohr radius $\hbar^2/me^2$. Specifi-

cally, it will be shown in this note that the factor $k \int fg \, dr$, through which the Dirac radial functions f, g and quantum number k enter into the relativistic hyperfine energy formula,[1] is equal to $(Z\alpha/2)<r^{-3}>$ where α is the fine structure constant $e^2/\hbar c$, Ze is the nuclear charge and the indicated expectation value is taken over the Dirac wave functions.

It is convenient to begin with the expression for the latter quantity $<r^{-3}>$. One has

$$(Ze^2/c)<r^{-3}> = \int_0^\infty r^2\psi^\dagger[(p_0 + \beta mc), \partial/\partial r]\psi \, dr \qquad (1)$$

where

$$p_0 = (W + Ze^2/r)/c \qquad (1.1)$$

and W is the energy, ψ and $\psi^\dagger$ are the Dirac wave function and its transposed complex conjugate, respectively, and β is the Dirac matrix ρ_3. Nothing essential is changed if Dirac's angle-dependent canonical transformation[2] is performed, which leads to the representation

$$\psi = \begin{pmatrix} f \\ g \end{pmatrix}, \psi^\dagger = (f, g), \epsilon = \begin{pmatrix} 0, & -i \\ i, & 0 \end{pmatrix},$$

$$\beta = \begin{pmatrix} 1, & 0 \\ 0, & -1 \end{pmatrix}, k = k\begin{pmatrix} 1, 0 \\ 0, 1 \end{pmatrix} \qquad (1.2)$$

A partial integration is then performed on the second term of the commutator, equation (1), and there results

$$(Ze^2/c)<r^{-3}> = \int 2r^2\psi^\dagger(p_0 + \beta mc)(d/dr + 1/r)\psi \, dr \qquad (1.3)$$

One can simplify this equation with the aid of the Hamiltonian equation, which can be expressed in the sense of equations (1.2) as

$$\{(p_0 + \beta mc) + (\epsilon \hbar/i)(d/dr + 1/r) + i\epsilon \beta k\hbar/r\}\psi = 0 \qquad (1.4)$$

If one multiplies equation (1.4) by

$$\psi^\dagger[(p_0 + \beta mc)i\epsilon/\hbar + \beta k/r]$$

from the left, then it is found that

$$\psi^\dagger[(p_0 + \beta mc) + \beta k \epsilon \hbar/ir](d/dr + 1/r)\psi = 0. \qquad (1.5)$$

Equation (1.3) then becomes

$$Z\alpha<r^{-3}> = \int 2rk[(d/dr)(fg) + 2fg/r]dr \qquad (1.6)$$

A partial integration is performed on the first term in the square brackets, and there results

$$(Z\alpha/2)<r^{-3}> = k \int fg \, dr.$$

It is a pleasure to acknowledge Professor Breit's interest and helpful advice concerning this work.

THE INFLUENCE OF SIZE OF TEST-FIELD SURROUND ON VISUAL INTENSITY DISCRIMINATION*

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The present report concerns a study of the effect of size of surround on the visual intensity discrimination threshold.

Steinhardt[1] has studied this problem during the course of an experiment that was primarily concerned with the relation between $\Delta I/I$ and intensity. His results are valuable but do not provide a complete analysis. In his experiments, the surround field brightness varied between 3.5 and 56 per cent of the standard brightness, I, on one half of the test field. Effects attributable to surround were greatest with small test fields. Other work has been done by Blachowski[2] and Fry and Bartley.[3] The Fry and Bartley experiment, in particular, contributes some important data and interpretations, but neither it nor the Blachowski study gives information on intensity discrimination thresholds over large ranges of adapting intensity. The present experiment attempts to do this. In addition, it provides, over the range of areas examined, a more detailed analysis than was feasible in the Steinhardt study. Intensity discrimination curves are obtained for adapting intensities varying over a range of 1 to 10,000 and for 15 combinations of differently sized test and background areas.

Method.—The apparatus, similar to one described by Baker,[4] presents the subject with a uniformly illuminated, circular field of light intensity, I, to which may be added, at intervals of one second, a momentarily exposed, circular field of illumination, ΔI. The fields are seen in "Maxwellian view." The intensity of illumination of the I-field is varied in steps over a wide range by decimal filters, and the illumination on the added ΔI-field is varied in small steps by a wedge in combination with filters. The diameters of both the I- and ΔI-fields may be varied in

regular steps (i.e., multiples of 2) by the provision of appropriately placed field stops with diameters of 1, 2, 4, 8 and 16 mm. These diameters provide visual fields of 0.6, 1.2, 2.4, 4.8 and 9.6 degrees at the subject's eye.

For any given combination of sizes of ΔI- and I-fields, an intensity discrimination curve is obtained by finding the threshold increment, ΔI_t, that is just perceptible at various levels of I. All combinations of I-field and ΔI-field that allow for a diameter of I-field greater than or equal to the diameter of the ΔI-field are used. The combinations range from one in which the I- and ΔI-fields are equal at a diameter of 0.6 degree to one in which both fields have a diameter of 9.6 degrees. Under all conditions, the ΔI-field, when it is added to the I-field in the form of a 0.02 second's flash, is centered on the latter. Since fifteen combinations of ΔI- and I-field exist, fifteen intensity discrimination curves were obtained for the subject of the experiment, Mr. Herschel Leibowitz. "White" light was used in all of the determinations, the filament lamps being operated on 110 volts d. c. The glass reflector used in Baker's study is replaced by one that is partially aluminized.

The brightnesses of the ΔI- and I-fields were determined by binocular match with an evenly illuminated surface viewed through an artificial pupil of the same size (2 mm diameter) as that provided in the eyepiece. Convergence was varied until the fields in the two eyes appeared side by side. With no filters in the beam of the adapting field, its apparent brightness was equal to that of a surface whose brightness (as measured by a Macbeth illuminometer) is 2830 millilamberts. Under the conditions of viewing through a circular artificial pupil of 2 mm diameter, this millilambert value is equivalent to a retinal brightness of 28,300 photons. [Photons $= (10/\pi) \times$ pupil area in square millimeters $\times$ brightness in millilamberts.] The ΔI-beam without filters, by the same type of measurement, gave an apparent brightness of 141,900 photons. Only one-tenth of the realizable brightness of the I-field was used as the maximum value in the experiments.

Before a given experimental session, the subject put on a pair of comfortably fitting red goggles[4] which he wore for thirty minutes before entering the darkroom. Once inside the darkroom, the subject took off the goggles and completed dark adaptation (to the limit of forty minutes) by remaining in the dark for ten minutes before making any observations. In the meantime the experimeter had placed the necessary filters in the optical system to provide the lowest level of adapting intensity for the particular surround area used in the particular session. In addition, provision had been made for the appropriate ΔI-field to be used on the given day. Following dark adaptation, the subject looked into the eyepiece and adapted for three minutes to the prevailing intensity, I. After the light adaptation interval the synchronous motor-sector disc shutter

was started and the subject reported on the presence or absence of a perceptible flash, ΔI. In any single determination of ΔI_t (i.e., the threshold value of ΔI) the method of limits was employed. The intensity of ΔI was varied in steps of 0.05 log unit, and two to four flashes were given at each step, the subject being required to tell whether or not he saw the added flash. In the ascending order, ΔI was increased in successive steps until the subject reported that he saw the flash; in the descending series, ΔI was decreased in steps until the subject stated that he did not see the flash. Five ascending and five descending series were used at each level of I to compute the threshold ΔI corresponding to the change in response.

Once ΔI_t had been determined for a given level of I, the experimeter changed the filters in the optical system, and the procedure of determining ΔI_t was repeated at an intensity level 10 times the initial one. In the
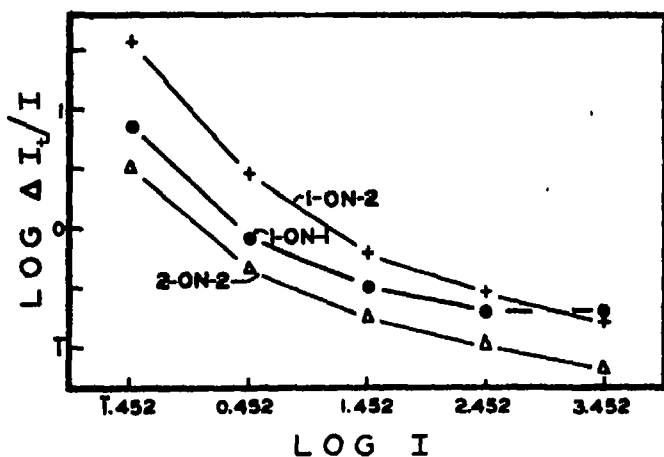
FIGURE 1

Log $\Delta I_t/I$ as a function of log I for three conditions of foveal ΔI- and I-fields. The labels on the curves refer to the diameters (in mm) of the ΔI- and I-fields, respectively.

course of a single day's determination, threshold values of ΔI were determined for five levels of I separated from each other by a logarithmic unit. All observations were made with monocular regard; the subject used his right eye. On successive days of experimentation various combinations of ΔI- and I-fields were employed, a single combination in a given experimental session. Fifteen such combinations were used, each of which eventuated in an intensity discrimination curve.

Results Obtained with the Two Smallest ΔI- and I-Fields.—The curves of figure 1, with labels representing field-stop diameters, represent results[*] obtained with ΔI- and I-fields that are restricted to the fovea. Each curve has the shape of the typical cone intensity discrimination curve.[7–9]

At a low intensity of adapting field, $\Delta I_t/I$ is large, and as the adapting intensity, I, increases, $\Delta I_t/I$ decreases until it approaches a final, limiting value at high intensities. The curve labeled 1-on-2 applies to the ΔI-field of 1 mm on an I-field of 2 mm; the 1-on-1 curve, to ΔI- and I-fields of 1 mm; and the 2-on-2 curve to ΔI- and I-fields of 2 mm. (A diameter, $D_{\Delta I}$ or D_I, of 1 mm represents a visual angle of 0.6°; a diameter of 2 mm, 1.2°.)

The results plotted for the 1-on-1 and 1-on-2 curves exhibit a striking effect. The 1-on-1 curve is shallow, and throughout most of its course, falls below the 1-on-2 curve. It cannot be superimposed on the 1-on-2 curve by an upward shift on the ordinate axis; for superposition, an additional shift along the abscissa axis is required.

The fact that the curve (1-on-2) for a small surround is lower than the curve (1-on-1) for no surround means that, when a small surround is present with a small ΔI-field, more added energy, ΔI, is required for intensity discrimination than is necessary when a surround is absent. In short, intensity discrimination for a small, foveal ΔI-field is, over a large range of adapting intensities, better with no surround than it is with a small surround.

The increase in threshold that is correlated with the presence of adjacent stimulation is analogous to a type of interaction effect often encountered in nervous centers, i.e., inhibition. The particular basis for the depressing or inhibitory effect encountered in the present experiments remains to be analyzed, but it is worth pointing out that similar threshold changes in the presence of adjacent stimulation have been reported for visual functions other than intensity discrimination.[10] The work of Fry and Bartley[3] demonstrates conditions for the lowering and raising of intensity discrimination thresholds under conditions where the test and surround configurations are complex.

The 2-on-2 curve ($D_{\Delta I} = D_I = 2$ mm $= 1.2°$) falls below the upper two curves of figure 1. This means that, so far as strictly foveal stimulation is concerned, discrimination is best with equally sized ΔI- and I-fields that approach the limits of the rod-free area of the retina. Within the same limits, an increase in the diameter of the ΔI-field results in better intensity discrimination. In the cases studied, an increase of a foveal I-field beyond the limits of the ΔI-field produces poorer discrimination.

Results Obtained with the Smallest ΔI-Field for All Sizes of I-Field.— Figure 2 gives all of the data of the experiment, but for the moment, we shall consider only the curves with crosses (i.e., those pertaining to the smallest ΔI-field) and note how they vary as D_I increases. Analysis is aided by observing the change in position of the curves containing crosses with respect to the upper of each pair of dashed-line curves in the various
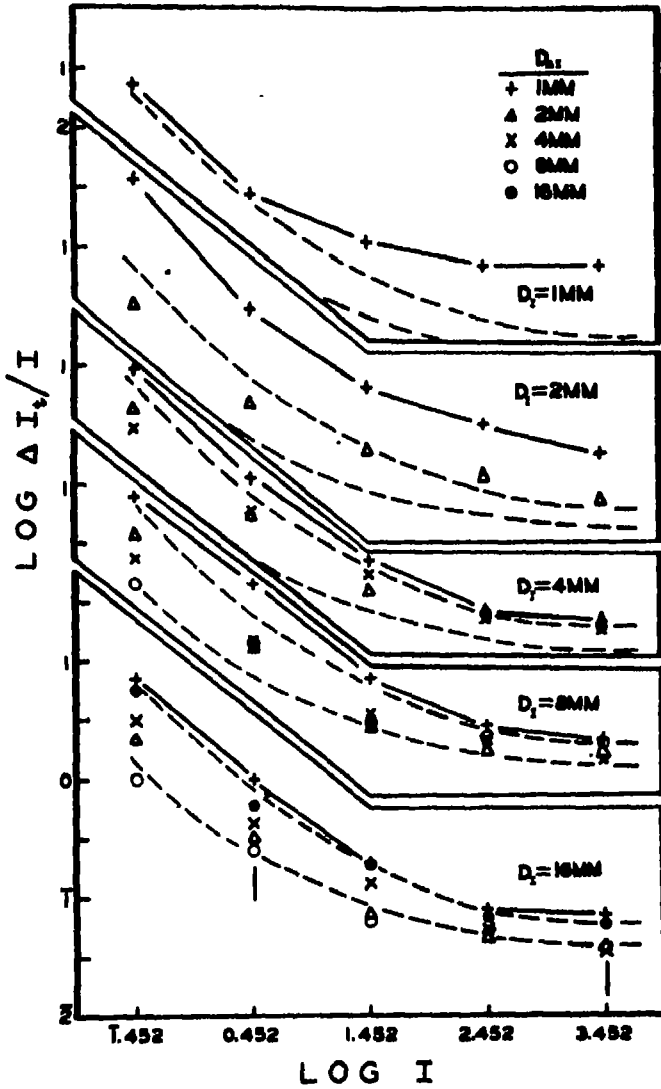
FIGURE 2

The complete data of the experiment. Each group of curves represents a given value of D_I. $D_{\Delta I}$ is a parameter that determines the position of each intensity discrimination curve within its D_I group. The dashed lines represent Hecht's equation[7] as described in the text.

sections of the graph. The corresponding dashed lines of the various sections are drawn through identical ordinate and abscissa values.

The rise in threshold found with a small surround does not occur with I-fields of 4 mm and greater. In fact, at high intensities, $\Delta I_t/I$ is smaller with large surrounds than it is when no surround is present. Figure 2 shows that $\Delta I_t/I$ reaches its minimum at high intensities and large values of I-field. A rise in threshold attributable to surround occurs only when the ΔI- and I-fields are restricted to the rod-free area. When I-field size exceeds this area, intensity discrimination for a small ΔI-field is improved. This result means that large I-fields provide effects[11] that "summate" with the processes due to ΔI. Whether the processes combine rod and cone effects remains a question.

Results Obtained with ΔI- and I-Fields Greater Than 1.2 Degrees.—Analysis of the results for ΔI- and I-fields that extend beyond the limits of the fovea may be made with the aid of figure 2, which presents all of the data of the experiment. In figure 2, each group of curves represents a given diameter, D_I, of I-field; the diameter of ΔI-field, $D_{\Delta I}$, is a parameter that determines the position of each intensity discrimination curve within a group of curves.

The lowest group of plotted points in figure 2, i.e., those for an I-field of 16 mm ($= 9.6$ degrees), contains two dashed-line curves (previously referred to) that set the limits of variation of the data for the four largest ΔI-fields. These curves, as drawn, represent Hecht's intensity discrimination equation, $\Delta I_t/I = c[1 + 1/(KI)^{1/4}]^2$, where c and K are constants.[7] The lower theoretical curve is fitted to the data for ΔI-fields of 2 ($= 1.2°$) and 8 mm ($= 4.8°$); the upper curve is the same curve moved to the right along the abscissa and upward on the ordinate in such a way as to fit the data for the ΔI-field of 16 mm ($= 9.6°$). The pair of curves representing Hecht's equation are repeated on the same coordinates in the data for the I-fields of 8, 4 and 2 mm. In general it may be said that Hecht's equation fits the data for the four largest ΔI-fields within the limits of experimental error, and it is probable that the same equation would fit the data for the smallest ΔI-field (for all values of D_I) if appropriate vertical and horizontal shifts were made.

Consider the data within the two theoretical curves of the lowest section of figure 2. Within the limits of variation set by the curves, the values of $\Delta I_t/I$ (at comparable values of I) are greatest for the largest ΔI-field (16 mm). The $\Delta I_t/I$ values are smaller for the ΔI-field of 4 mm, and then successively smaller for the ΔI-fields of 2 and 8 mm. Thus, it may be said that curve position is not an obvious parameter of size of ΔI-field; reversals in curve order occur.

The reversal in order of the curves is shown in figure 3 which plots the data for $\log I = 0.452$ and $\log I = 3.452$ of the present experiment. (The

data are represented by filled circles in figure 3 and constitute what is called the Regular Series; the vertical lines in figure 2 denote the selected adapting intensities.)   In figure 3 the curves of log $\Delta I_t/I$ for various values of D_I show, at the two intensities used, a jagged appearance.   At both intensities, $\Delta I_t/I$ is highest for the ΔI-field of 1 mm.   The curves traced through the points for the larger fields are not regular, and at both adapting intensities, $\Delta I_t/I$ for the largest ΔI-field (16 mm) is higher than it is for fields of 2, 4 and 8 mm.   The data, then, do not show a systematic variation of $\Delta I_t/I$ with diameter of ΔI-field.

In an experiment of the type described it may be expected that day-to-day variances will contribute to the variability of the experimental results.
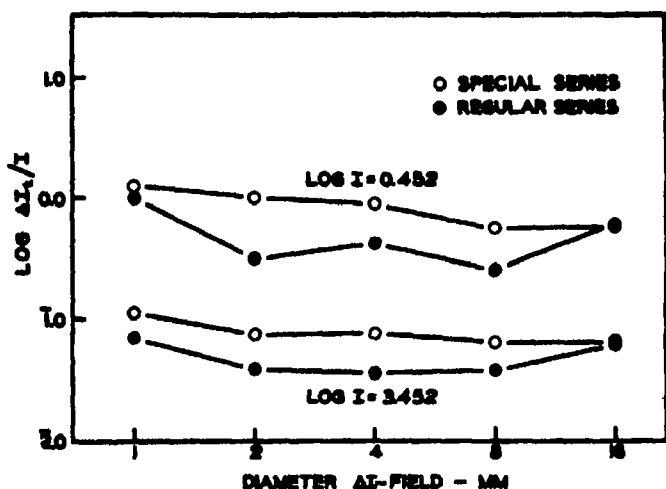
FIGURE 3

The intensity discrimination ratio as a function of the diameter of ΔI-field at two levels of I in two experiments.   In the Regular Series, day-to-day variances are large; in the Special Series, they are minimized.   The diameter of I-field is 16 mm.

Because of this fact a separate experiment was performed at the I values indicated by the vertical lines of figure 2.   The results of this special series of determinations are plotted as open circles in figure 3.   Since all of the values for a given adapting intensity were obtained in a single session (with an I-field of 16 mm) it may be expected that day-to-day variances will be minimized.   The curves obtained in single sessions (i.e., the Special Series curves) are, in fact, more regular than the curves (Regular Series) obtained from the data of different days. ·

· In the Special Series, $\Delta I_t/I$ for the smallest diameter of ΔI-field is larger in both curves than it is for the four largest diameters of ΔI-field.   The curve for log I = 3.452 shows no change in $\Delta I_t/I$ for the four largest

diameters, and the decrease shown in the curve for log $I = 0.452$ is small; it covers a range of about 0.2 log unit. Since the effect is so small as to be readily masked by day-to-day variances, it may be thought of as a second order effect within the range of areas encountered in this experiment.

The dashed line curves drawn about the data for I-fields of 8, 4 and 2 mm are identical with the theoretical curves that apply to the data of the largest I-field. All pairs of theoretical curves seem to embrace their respective data in a comparable manner. This result, together with previous considerations as to variability, indicates that, for I-fields greater than 1.2 degrees ($= 2$ mm), $\Delta I_t/I$ (at comparable adapting intensities) remains unchanged with an increase in the size of the surrounding I-field. In addition, it means that an increase in the size of the ΔI-field beyond about 1.2 degrees has, at best, a small effect on the magnitude of $\Delta I_t/I$.

The conclusion of the last paragraph may not be surprising when it is remembered that the curves of figures 1 and 2 are ordinarily accepted as curves of cone function. Increasing the diameters of the I-field and the ΔI-field beyond the retinal limits where cone functions predominate does not change the value of $\Delta I_t/I$ at intensities where the cones are the basic determiners of discrimination.

Summary.—(1) A method is described whereby a subject is stimulated by a uniformly illuminated circular field of brightness, I, to which may be added, at intervals of one second, a momentarily illuminated, circular field of brightness, ΔI. Threshold values of ΔI are obtained as a function of I for 15 combinations of sizes of ΔI- and I-field. The largest visual field used has a diameter of 9.6 degrees. The intensity values chosen provide data on the "cone" portion of the intensity discrimination curve. (2) Intensity discrimination for a small, foveal ΔI-field is, over a large range of adapting intensities, better with no surround than it is with a small surround. When surround size increases beyond the limits of the rod-free area, the intensity discrimination threshold for the small ΔI-field decreases, at high intensities, below the value obtained with no surround. These results are interpreted in terms of a concept of interaction. (3) $\Delta I_t/I$ values that lie on the cone portion of the intensity discrimination curve are lowered only slightly and uncertainly by increases in the diameter of either the ΔI- or I-field beyond the limits of a central area of about 1.2 degrees.

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HOMOGENEOUS CONTACT TRANSFORMATIONS

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A necessary and sufficient condition that

$$\bar{x}^i = \varphi^i(x^1, x^2, \ldots, x^n; \quad p_1, p_2, \ldots, p_n),$$
$$\bar{p}_i = \psi_i(x^1, x^2, \ldots, x^n; \quad p_1, p_2, \ldots, p_n) \tag{1}$$

be the equations of a homogeneous contact transformation is that $\bar{x}^i$ and $\bar{p}_i$ satisfy the equations[1]

$$\bar{p}_i \frac{\partial \bar{x}^i}{\partial x^\alpha} = p_\alpha, \qquad \bar{p}_i \frac{\partial \bar{x}^i}{\partial p_\alpha} = 0 \quad (\alpha = 1, \ldots, n). \tag{2}$$

In these equations we use the convention, to be used throughout this paper, that when the same index appears twice in a term this term stands for the sum of the terms obtained by giving the index each of its values.

From equations (2) it can be shown that $\bar{x}^i$ are functions homogeneous of degree zero in the p's and that $\bar{p}_i$ are homogeneous of degree one in the p's.

We consider now the system of differential equations

$$X^\beta f = \frac{\partial f}{\partial p^\beta} + h^{\beta\alpha} \frac{\partial f}{\partial x^\alpha} = 0 \quad (\alpha, \beta = 1, \ldots, n). \tag{3}$$

Since these equations are in Jacobian form, if they are to admit n independent solutions $\bar{x}^i$, the commutator $(X^\gamma, X^\beta)f$ must be identically zero,[2] that is

$$h^{\gamma\delta} \frac{\partial h^{\beta\alpha}}{\partial x^\delta} - h^{\beta\delta} \frac{\partial h^{\gamma\alpha}}{\partial x^\delta} + \frac{\partial h^{\beta\alpha}}{\partial p_\gamma} - \frac{\partial h^{\gamma\alpha}}{\partial p_\beta} = 0 \quad (\alpha, \beta, \gamma, \delta = 1, \ldots, n). \tag{4}$$

For n independent solutions of (3) the rank of the matrix of the quantities $\partial \bar{x}^i/\partial x^\alpha$ and $\partial \bar{x}^i/\partial p_\alpha$ is n. Since it follows from (3) that any determinant of order n of this matrix is a multiple of the determinant of the quantities $\partial \bar{x}^i/\partial x^\alpha$, we have that this determinant is not identically zero.

If now the solutions $\bar{x}^i$ of equations (3) are to be the first set of equations (1) of a homogeneous contact transformation, we note from the second of equations (2) that the matrix of the quantities $\partial \bar{x}^i/\partial p_\alpha$ is of rank less than n. Then from (3) and the fact that the determinant of the quantities $\partial \bar{x}^i/\partial x^\alpha$ is not zero it follows that the matrix of the quantities $h^{\beta\alpha}$ is of rank less than n. Since the $\bar{x}^i$ are to be homogeneous of degree zero in the p's, if equation (3) in $\bar{x}^i$ is multiplied by p_β and summed for β, we obtain

$$p_\beta h^{\beta\alpha} = 0. \tag{5}$$

From the second of (2) and (3) we have

$$p_i h^{\beta\alpha} \frac{\partial \bar{x}^i}{\partial x^\alpha} = 0,$$

from which and the first of (2) we obtain

$$p_\alpha h^{\beta\alpha} = 0. \tag{6}$$

By differentiation of this equation we obtain

$$p_\alpha \frac{\partial h^{\beta\alpha}}{\partial x^\delta} = 0, \qquad p_\alpha \frac{\partial h^{\beta\alpha}}{\partial p_\gamma} + h^{\beta\gamma} = 0. \tag{7}$$

By means of these equations, if we multiply equation (4) by p_α and sum for α, we obtain $h^{\beta\gamma} = h^{\gamma\beta}$, that is the quantities $h^{\beta\alpha}$ are symmetric in their indices.

We consider now the system of partial differential equations[3]

$$\frac{\partial x^\alpha}{\partial p_\beta} = h^{\alpha\beta}. \tag{8}$$

A necessary and sufficient condition that these equations be completely integrable, that is, that there be a solution involving n independent constants is the same as the condition that the associated system (3) admit n independent solutions,[3] that is, equations (4).

In consequence of (6) and (8) we have $p_\beta(\partial x^\alpha/\partial p_\beta) = 0$, that is, the solutions x^α of equation (8) are homogeneous of degree zero in the p's.

Since the quantities $h^{\alpha\beta}$ are symmetric in their indices, we have from (8) that

$$\frac{\partial x^\alpha}{\partial p_\beta} = \frac{\partial x^\beta}{\partial p_\alpha} \tag{9}$$

Put

$$x^1 = \frac{\partial \varphi}{\partial p_1} + f^1(c^1, \ldots, c^n),$$

where φ is a non-linear homogeneous function of the p's of degree one as follows from (8), and $c^1, \ldots, c^n$ are constants. Substituting in equation (9) for $\alpha = 1$, $\beta = 2$, we obtain by integration

$$x^2 = \frac{\partial \varphi}{\partial p_2} + \frac{\partial \psi}{\partial p_2} + f^2(c^1, \ldots, c^n),$$

where ψ does not involve p_1 and is homogeneous of degree one. From the form of the above expression for x^1 we have that ψ can be included in φ, so that in all generality we have

$$x^2 = \frac{\partial \varphi}{\partial p_2} + f^2(c^1, \ldots, c^n).$$

For $\alpha = 1, 2$, and $\beta = 3$ in (9) we have by integration

$$x^3 = \frac{\partial \varphi}{\partial p_3} + \frac{\partial \sigma}{\partial p_3} + f^3(c^1, \ldots, c^n),$$

where σ does not involve p_1 and p_2, is homogeneous of degree one, and consequently can be included in φ. Continuing this process we note that in all generality we have

$$x^\alpha = \frac{\partial \varphi}{\partial p_\alpha} + f^\alpha(c^1, \ldots, c^n) \quad (\alpha = 1, \ldots, n) \tag{10}$$

where the functions f^α are independent.

From these equations and (8) we have

$$h^{\alpha\beta} = \frac{\partial^2 \varphi}{\partial p_\alpha \partial p_\beta}, \tag{11}$$

with the result that equations (4) are satisfied, and also (5) and (6).

In accordance with the theory of equations (8) and the associated system (3), if $\theta^\alpha(x, p) = c^\alpha$ are solutions of equations (10), the functions θ^α are n independent solutions of equations[3].

In the present case the equations

$$\bar{x}^i = \psi^i(y^1, \ldots, y^n), \text{ where } y^\alpha = x^\alpha - \frac{\partial \varphi}{\partial p^\alpha}, \tag{12}$$

ψ^i being any independent function of the y's, are the first set of equations of a homogeneous contact transformation. For the determination of the second set we have from (2)

$$\bar{p}_i \frac{\partial \psi^i}{\partial y^\alpha} = p_\alpha, \qquad \bar{p}_i \frac{\partial \psi^i}{\partial y^\beta} \frac{\partial^2 \varphi}{\partial p_\alpha \partial p_\beta} = 0. \tag{13}$$

Since φ is by hypothesis homogeneous of degree one in the p's, the unique solution of the first set of these equations satisfies the second set. Hence we have:

. If φ is any non-linear function of $p_1, \ldots, p_n$ homogeneous of degree one, and ψ^i are any n independent functions of $x^1 - \partial\varphi/\partial p_1, \ldots, x^n - \partial\varphi/\partial p_n$, the equations $\bar{x}^i = \psi^i$ are the first set of equations of a homogeneous contact transformation, and the second set is obtained by solving the first set of equations (13) where $y^\alpha = x^\alpha - \partial\varphi/\partial p_\alpha$.

We consider next the case when the matrix of the quantities $\partial\bar{x}^i/\partial x^\alpha$ for $\alpha = 1, \ldots, n$ is of rank less than n, and assume that the matrix of the quantities $\partial\bar{x}^i/\partial x^\alpha$ for $\alpha = 1, \ldots, r(>1)$ and $\partial\bar{x}^i/\partial p_\lambda$ for $\lambda = r + 1, \ldots, n$ is of rank n. In place of equations (3) we consider the system

$$X_\mu f \equiv \frac{\partial f}{\partial x^\mu} + h_\mu{}^\alpha \frac{\partial f}{\partial x^\alpha} + h_{\mu\lambda} \frac{\partial f}{\partial p_\lambda} = 0,$$

$$X^\beta f \equiv \frac{\partial f}{\partial p_\beta} + h^{\beta\alpha} \frac{\partial f}{\partial x^\alpha} + h^\beta{}_\lambda \frac{\partial f}{\partial p_\lambda} = 0, \tag{14}$$

$$(\alpha, \beta = 1, \ldots, r; \ \lambda, \mu = r + 1, \ldots, n),$$

and assume that they admit n independent solutions $\bar{x}^i = \varphi^i(x, p)$, which are the first set of equations (1).

If these equations in $\bar{x}^i$ are multiplied by $\bar{p}_i$ and summed for i and equations (2) are to be satisfied, we have,

$$h_\mu{}^\alpha p_\alpha + p_\mu = 0, \qquad h^{\beta\alpha} p_\alpha = 0. \tag{15}$$

Since $\bar{x}^i$ are to be homogeneous of degree zero in the p's,

$$p_\beta \frac{\partial \bar{x}^i}{\partial p_\beta} + p_\lambda \frac{\partial \bar{x}^i}{\partial p_\lambda} = 0,$$

by means of which we have from the second of (14) in $\bar{x}^i$

$$p_\beta h^{\beta\alpha} \frac{\partial \bar{x}^i}{\partial x^\alpha} + (p_\beta h^\beta{}_\lambda - p_\lambda) \frac{\partial \bar{x}^i}{\partial p_\lambda} = 0.$$

Consequently

$$p_\beta h^{\beta\alpha} = 0, \qquad p_\beta h^\beta{}_\lambda = p_\lambda, \tag{16}$$

since the matrix of $\partial\bar{x}^i/\partial x^\alpha$ and $\partial\bar{x}^i/\partial p_\lambda$ is of rank n by hypothesis.

The equations for which equations (14) are the associated system are

$$\frac{\partial x^{\alpha}}{\partial x^{\mu}} = h_{\mu}{}^{\alpha}, \qquad \frac{\partial p_{\lambda}}{\partial x^{\mu}} = h_{\mu\lambda}, \tag{17}$$

$$\frac{\partial x^{\alpha}}{\partial p_{\beta}} = h^{\beta\alpha}, \qquad \frac{\partial p_{\lambda}}{\partial p_{\beta}} = h^{\beta}{}_{\lambda}. \tag{18}$$

The conditions of integrability of the first set of (18) are

$$\frac{\partial h^{\beta\alpha}}{\partial x^{\delta}} h^{\gamma\delta} - \frac{\partial h^{\gamma\alpha}}{\partial x^{\delta}} h^{\beta\delta} + \frac{\partial h^{\beta\alpha}}{\partial p_{\gamma}} - \frac{\partial h^{\gamma\alpha}}{\partial p_{\beta}} + \frac{\partial h^{\beta\alpha}}{\partial p_{\lambda}} h^{\gamma}{}_{\lambda} - \frac{\partial h^{\gamma\alpha}}{\partial p_{\lambda}} h^{\beta}{}_{\lambda} = 0 \tag{19}$$

$$(\alpha, \beta, \gamma, \delta = 1, \ldots, r; \ \lambda = r + 1, \ldots, n).$$

From the second of equations (15) we have

$$p_{\alpha} \frac{\partial h^{\beta\alpha}}{\partial x^{\delta}} = 0, \qquad p_{\alpha} \frac{\partial h^{\beta\alpha}}{\partial p_{\gamma}} + h^{\beta\gamma} = 0, \qquad p_{\alpha} \frac{\partial h^{\beta\alpha}}{\partial p_{\lambda}} = 0.$$

If equation (19) is multiplied by p_{α} and α is summed, we have in consequence of the above equations that $h^{\beta\gamma}$ is symmetric in its indices.

Making use of this fact and proceeding with the first of equations (18) as was done with equation (9) we take

$$x^{\alpha} = \frac{\partial \varphi}{\partial p_{\alpha}} + c^{i}\psi_{i}{}^{\alpha}(x^{r+1}, \ldots, x^{n}), \tag{20}$$

where φ is any non-linear function of $p_{1}, \ldots, p_{r}$ homogeneous of degree one. From the first of equations (15) and (17) we have

$$p_{\lambda} = -p_{\alpha} \frac{\partial x^{\alpha}}{\partial x^{\lambda}} = -c^{i} p_{\alpha} \frac{\partial \psi_{i}{}^{\alpha}}{\partial x^{\lambda}}. \tag{21}$$

If then the functions $\psi_{i}{}^{\alpha}$ are such that the determinant D of the quantities $\psi_{i}{}^{\alpha}$ and $p_{\alpha}(\partial\psi_{i}{}^{\alpha}/\partial x^{\lambda})$ is different from zero, we have on solving equations (20) and (21) for the c's

$$c^{i} = \theta^{i}(x, p) = \left(x^{\alpha} - \frac{\partial \varphi}{\partial p_{\alpha}}\right) \bar{\psi}_{\alpha}{}^{i} - p_{\lambda} \bar{\psi}^{\lambda i}, \tag{22}$$

where $\bar{\psi}_{\alpha}{}^{i}$ and $\bar{\psi}^{\lambda i}$ are the cofactors of $\psi_{i}{}^{\alpha}$ and $p_{\alpha}(\partial\psi_{i}{}^{\alpha}/\partial x^{\lambda})$, respectively, in D divided by D. Accordingly $\bar{\psi}_{\alpha}{}^{i}$ and $\bar{\psi}^{\lambda i}$ are homogeneous of degrees 0 and -1, respectively, in $p_{1}, \ldots, p_{r}$, and consequently θ^{i} are homogeneous of degree zero in $p_{1}, \ldots, p_{n}$.

In accordance with the general theory[3] of systems of equations of the form (17) and (18) and the associated system (14), the functions θ^{i} in (22) are solutions of equations (14). So also is any function of the θ's. Hence

$$\bar{x}^i = f^i(\theta^1, \ldots, \theta^n), \tag{23}$$

where f^i are any n independent functions of the θ's, are the first set of equations of a homogeneous contact transformation. Equations (2) are satisfied by $\bar{p}_i$ obtained by solving the equations

$$\bar{p}_i \frac{\partial f^i}{\partial \theta^j} = p_\beta \psi_j^\beta. \tag{24}$$

Hence we have:

Given any non-linear function φ of $p_1, \ldots, p_r$ homogeneous of degree one in the p's and functions $\psi_i^\alpha(x^{r+1}, \ldots, x^n)$, for $\alpha = 1, \ldots, r, i = 1, \ldots, n$, such that the determinant D of ψ_i^α and $p_\alpha \partial \psi_i^\alpha / \partial x^\lambda$ for $\lambda = x^{r+1}, \ldots, x^n$, is not zero; a homogeneous contact transformation is defined by equations (23) and (24), where f^i are any n independent functions of the θ's, the latter defined by (22) where $\bar{\psi}_\alpha^i$ and $\bar{\psi}^{\lambda i}$ are the cofactors of ψ_i^α and $p_\alpha(\partial \psi_i^\alpha / \partial x^\lambda)$, respectively, in D divided by D.

There remains for consideration the case where the matrix of $\partial \bar{x}^i / \partial x^\alpha$ is of rank one. In this case we have equations (14), (17), (18) for $\alpha, \beta = 1$. $\lambda, \mu = 2, \ldots, n$, where

$$h_\mu{}^1 = -\frac{p_\mu}{p_1}, \qquad h_{\mu\lambda} = 0, \qquad h^{11} = 0, \qquad h^1{}_\lambda = \frac{p_\lambda}{p_1}.$$

In place of (22) we have

$$c^i = \theta^i(x, p) = \frac{1}{p_1}(b^i x^\gamma p_\gamma - b^{i\lambda} p_\lambda) \qquad (\gamma = 1, \ldots, n; \lambda = 2, \ldots, n),$$

$$\tag{25}$$

where b^i and $b^{i\lambda}$ are constants whose matrix is of rank n. For this expression for θ^i equations (23) are the first set of equations of a homogeneous contact transformation and the second set is obtained by solving the equations

$$\bar{p}_i \frac{\partial f^i}{\partial \theta^j} = \left(\frac{\bar{b}_j}{n} + \bar{b}_{j\mu} x^\mu\right) p_1 \qquad (\mu = 2, \ldots, n),$$

where $\bar{b}_j$ and $\bar{b}_{j\mu}$ are the cofactors of b^j and $b^{j\mu}$ in the determinant of these quantities divided by the determinant.

[1] *C. G.*, pp. 239, 240. A reference of this type is to the author's *Continuous Groups of Transformations*, Princeton University Press, 1933. The geometric properties of these transformations, accounting for the term contact, are derived in pp. 242–245. This material may also be found in the author's article "Contact Transformations," *Ann. Math.*, 30, 211–249 (1929).

[2] *C. G.*, p. 8.

[3] Cf. *C. G.*, pp. 3–5.

ON A THEOREM OF WEYL CONCERNING EIGENVALUES OF LINEAR TRANSFORMATIONS. II*

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1. This is the continuation of an earlier Note.[1] The main result of the present Note is a generalization of Weyl's theorem[2] which has been restated at the beginning of I. Let A be either a linear transformation in an n-dimensional unitary space or a completely continuous linear operator in Hilbert space. Let λ_i be the successive eigenvalues of A and κ_i be those of the non-negative Hermitian transformation A^*A. Weyl's theorem gives a set of inequalities comparing $|\lambda_i|^2$ with κ_i. Our generalization will show that Weyl's inequalities remain valid if the transformation A^*A is replaced by the transformation $\beta A^*A + \gamma AA^*$, where β, γ are any two non-negative numbers with $\beta + \gamma = 1$.

We shall also consider the Hermitian transformation $(A + A^*)/2$ and give a set of inequalities comparing its eigenvalues with the real parts $\Re \lambda_i$ of the eigenvalues λ_i of A.

In the following we shall only deal with linear transformations in a finite-dimensional unitary space. Nevertheless it will become clear that both Theorems 1 and 2 and their proofs can be easily carried over to completely continuous linear operators in Hilbert space, especially to linear integral equations with continuous kernels.

I am greatly indebted to Professor H. Weyl for his valuable criticisms which led to the final form and generality in which the results are presented here.

2. We begin with the following lemma which may be regarded as a matric generalization of the inequality between the arithmetic and geometric means.

LEMMA 1. *Let B and C be two n-rowed non-negative Hermitian matrices and let β, γ be two non-negative numbers with $\beta + \gamma = 1$. Then*

$$\det(\beta B + \gamma C) \geq (\det B)^\beta (\det C)^\gamma. \tag{1}$$

Obviously we need only to consider the case where B, C are both positive definite. Then there exists a non-singular matrix T such that T^*BT and T^*CT are both of diagonal form. Let the diagonal-elements of T^*BT and T^*CT be μ_i and $\nu_i (1 \leq i \leq n)$, respectively. These numbers μ_i, ν_i are necessarily > 0. The matrix $T^*(\beta B + \gamma C)T$ is also of diagonal form with diagonal-elements $\beta \mu_i + \gamma \nu_i (1 \leq i \leq n)$. It is clear that inequality (1) is equivalent to the trivial inequality

$$\prod_{i=1}^{n} (\beta\mu_i + \gamma\nu_i) \geq \prod_{i=1}^{n} \mu_i^{\beta}\nu_i^{\gamma}.$$

LEMMA 2. *If A is an arbitrary linear transformation in the n-dimensional unitary space and e_i $(1 \leq i \leq n)$ are n orthonormal vectors in the space, then for $q = 1, 2, \ldots, n$:*

$$\left| \det(Ae_i, e_j)_{i, j = 1, \ldots, q} \right|^2 \leq \det(Ae_i, Ae_j)_{i, j = 1, \ldots, q} \qquad (2)$$

$$\left| \det(Ae_i, e_j)_{i, j = 1, \ldots, q} \right|^2 \leq \det(A^*e_i, A^*e_j)_{i, j = 1, \ldots, q}. \qquad (3)$$

In fact, if we take e_i $(1 \leq i \leq n)$ as the unit vectors and denote the q vectors Ae_i by $x_i = \sum_{j=1}^{n} x_{ij}e_j = Ae_i$ $(1 \leq i \leq q)$, then inequality (2) reads

$$\text{abs.}^2 \begin{vmatrix} x_{11}x_{12}\ldots x_{1q} \\ x_{21}x_{22}\ldots x_{2q} \\ \cdot \cdot \cdot \cdot \cdot \\ x_{q1}x_{q2}\ldots x_{qq} \end{vmatrix} \leq \begin{vmatrix} (x_1, x_1)(x_1, x_2)\ldots(x_1, x_q) \\ (x_2, x_1)(x_2, x_2)\ldots(x_2, x_q) \\ \cdot \cdot \cdot \cdot \cdot \cdot \cdot \cdot \\ (x_q, x_1)(x_q, x_2)\ldots(x_q, x_q) \end{vmatrix}$$

which is evident, since the Gram determinant on the right side equals

$$\sum_{1 \leq i_1 < i_2 < \ldots < i_q \leq n} \text{abs.}^2 \begin{vmatrix} x_{1i_1}x_{1i_2}\ldots x_{1i_q} \\ x_{2i_1}x_{2i_2}\ldots x_{2i_q} \\ \cdot \cdot \cdot \cdot \cdot \cdot \\ x_{qi_1}x_{qi_2}\ldots x_{qi_q} \end{vmatrix}$$

As the matrices $\|(Ae_i, e_j)\|$ and $\|(A^*e_i, e_j)\|$ $(i, j = 1, 2, \ldots, q)$ are Hermitian conjugates of each other, inequality (3) is obtained by applying inequality (2) to A^*.

LEMMA 3. *Let H be a non-negative Hermitian transformation in the n-dimensional unitary space. If the eigenvalues $\xi_i (1 \leq i \leq n)$ of H are arranged in descending order, then for any positive integer $q \leq n$, the product $\prod_{i=1}^{q} \xi_i$ is the maximum of the q-rowed determinant $\det (He_i, e_j)_{i, j=1, \ldots, q}$, when q orthonormal vectors $e_1, \ldots, e_q$ vary.*

We need only to show that the inequality

$$\prod_{i=1}^{q} \xi_i \geq \det(He_i, e_j)_{i, j=1, \ldots, q} \qquad (4)$$

holds for any q orthonormal vectors $e_1, \ldots, e_q$. The case $q = 1$ is well known. For an arbitrary value q, we apply the case $q = 1$ of relation (4) to the linear transformation $H^{[q]}$ induced by H for the space elements (skew-symmetric tensors) of rank q.

We come now to our main result:

THEOREM 1. (*Generalisation of Weyl's Theorem.*) *Let A be an arbitrary*

linear transformation in the n-dimensional unitary space, and let β, γ be two non-negative-numbers with $\beta + \gamma = 1$. Let the eigenvalues of the transformation A and the non-negative Hermitian transformation $H = \beta A^*A + \gamma A A^*$ be denoted by λ_i and ξ_i $(1 \leq i \leq n)$, respectively, which are so arranged that

$$|\lambda_1| \geq |\lambda_2| \geq \ldots \geq |\lambda_n|, \qquad \xi_1 \geq \xi_2 \geq \ldots \geq \xi_n. \qquad (5)$$

Then for any non-decreasing function $\omega(t)$ on $t \geq 0$ such that $\omega(e^\tau)$ is a convex function of τ, the inequalities

$$\sum_{i=1}^{q} \omega(|\lambda_i|^2) \leq \sum_{i=1}^{q} \omega(\xi_i) \qquad (1 \leq q \leq n) \qquad (6)$$

hold. In particular, for any real exponent $s > 0$:

$$\sum_{i=1}^{q} |\lambda_i|^{2s} \leq \sum_{i=1}^{q} \xi_i^s \qquad (1 \leq q \leq n). \qquad (7)$$

As in Weyl's proof of his theorem, we need only to establish inequalities

$$\prod_{i=1}^{q} |\lambda_i|^2 \leq \prod_{i=1}^{q} \xi_i. \qquad (1 \leq q \leq n). \qquad (8)$$

To derive relation (6) from relation (8), one can either use Weyl's lemma[2] or apply a lemma due to G. Pólya[3] to the two sets of numbers $\log |\lambda_i|^2$ and $\log \xi_i$.

Since $\lambda_i (1 \leq i \leq n)$ are eigenvalues of A, there exist n orthonormal vectors $e_1, \ldots, e_n$ such that $(Ae_i, e_i) = \lambda_i (1 \leq i \leq n)$ and $(Ae_i, e_j) = 0$ for $1 \leq i < j \leq n$. Set

$$||(Ae_i, Ae_j)||_{i, j=1, \ldots, q} = B_q, \qquad ||(A^*e_i, A^*e_j)||_{i, j=1, \ldots, q} = C_q.$$

Then Lemma 2 yields inequalities

$$\prod_{i=1}^{q} |\lambda_i|^2 \leq \det B_q, \qquad \prod_{i=1}^{q} |\lambda_i|^2 \leq \det C_q.$$

Consequently

$$\prod_{i=1}^{q} |\lambda_i|^2 \leq (\det B_q)^\beta (\det C_q)^\gamma$$

and according to Lemma 1 the right side is less than or equal to $\det H_q$, where $H_q = \beta B_q + \gamma C_q$ is the matrix $||(He_i, e_j)||_{i, j=1, \ldots, q}$. On the other hand, Lemma 3 asserts that

$$\det H_q \leq \prod_{i=1}^{q} \xi_i.$$

Thus (8) is proved.

3. Denote now by $\kappa_i (1 \leq i \leq n)$ the eigenvalues of A^*A arranged in descending order. Between the eigenvalues ξ_i of $H = \beta A^*A + \gamma AA^*$ (for an arbitrary pair $\beta \geq 0$, $\gamma \geq 0$, $\beta + \gamma = 1$) and the eigenvalues κ_i of A^*A, inequalities

$$\sum_{i=1}^{q} \xi_i \leq \sum_{i=1}^{q} \kappa_i \qquad (1 \leq q \leq n) \tag{9}$$

hold. In fact, by Theorem 1 of I, the left side is the maximum of

$$\sum_{i=1}^{q} (Hy_i, y_i) = \beta \sum_{i=1}^{q} (A^*Ay_i, y_i) + \gamma \sum_{i=1}^{q} (AA^*y_i, y_i),$$

when q orthonormal vectors $y_1, \ldots, y_q$ vary. This maximum is not greater than

$$\beta \underset{y_i}{\operatorname{Max}} \sum_{i=1}^{q} (A^*Ay_i, y_i) + \gamma \underset{z_i}{\operatorname{Max}} \sum_{i=1}^{q} (AA^*z_i, z_i).$$

The last two maxima are both equal to the right side of relation (9). From formula (9) and Pólya's lemma quoted above, one concludes that inequalities

$$\sum_{i=1}^{q} \Omega(\xi_i) \leq \sum_{i=1}^{q} \Omega(\kappa_i) \qquad (1 \leq q \leq n) \tag{10}$$

are satisfied for any non-decreasing convex function $\Omega(t)$. Of course this class of functions $\Omega(t)$ is much narrower than the class of functions $\omega(t)$ figuring in Weyl's theorem as well as in the above generalized theorem.

4. One would naturally compare the real parts $\Re\lambda_i$ of the eigenvalues λ_i of A with the eigenvalues ρ_i of the Hermitian transformation $(A + A^*)/2$. For this comparison, we have

THEOREM 2. *Let A be an arbitrary linear transformation in the n-dimensional unitary space. If the eigenvalues λ_i, $\rho_i (1 \leq i \leq n)$ of A and $(A + A^*)/2$, respectively, are so arranged that*

$$\Re\lambda_1 \geq \Re\lambda_2 \geq \ldots \geq \Re\lambda_n, \qquad \rho_1 \geq \rho_2 \geq \ldots \geq \rho_n, \tag{11}$$

then they satisfy the inequalities

$$\sum_{i=1}^{q} \Omega(\Re\lambda_i) \leq \sum_{i=1}^{q} \Omega(\rho_i) \qquad (1 \leq q \leq n) \tag{12}$$

for any non-decreasing convex function $\Omega(t)$.

First the case $\Omega(t) = t$ can be easily proved by using Theorem 1 of I. Then the general case follows by applying Pólya's lemma.

Obviously, inequalities similar to formula (12) exist between the imaginary parts of λ_i and the eigenvalues of the Hermitian transformation $(A - A^*)/2\sqrt{-1}$.

A COMMUTATIVITY THEOREM FOR NORMAL OPERATORS

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1. Introduction.—This note contains two results concerning linear operators in Hilbert space $\mathfrak{H}$.

THEOREM I: *Let B be a bounded[1] operator and N a normal but not necessarily bounded operator with the canonical spectral representation*

$$N = \int z\,dE_z \qquad (z = x + iy).$$

Suppose that B commutes with N:

$$BN \subseteqq NB.$$

Then B commutes with E_z for any z: $BE_z = E_z B$ and hence B commutes with any function of N, e.g.

$$BN^* \subseteqq N^*B \qquad (or\ B^*N \subseteqq NB^*).$$

This theorem is very easily obtained in the case where N has a pure point spectrum. In the general case we may approximate N by operators with pure point spectra. Although these approximating operators in general do not commute with B, it turns out that the proof can be carried through along these lines, as shown in section 2. A second proof has later on been established by P. R. Halmos.

It is still an open question whether or not $NT \subseteqq TN$ implies $N^*T \subseteqq TN^*$ if N is bounded and normal and T is closed but non-bounded. In the case of two non-bounded operators the concept of commutativity is not even generally defined. It is, nevertheless, worth while to mention an example of two non-bounded, normal operators N_1 and N_2 which in a very suggestive way behave like commuting operators whereas N_1 and N_2^* behave like non-commuting operators. The example was constructed first by J. v. Neumann,[2] p. 61, footnote 37.

In section 3 we will discuss a conversion of Theorem I.

2. Proof of Theorem I.—We introduce an arbitrary square lattice with lines parallel to the coördinate axes of the complex plane. The length of

the sides of the squares is called s. The squares are considered as closed at left and below so that they are mutually disjoint. We arrange them as a sequence $\sigma_1, \sigma_2, \ldots, \sigma_n, \ldots$. The center of σ_i is called s_i. For any Borel set α in the complex plane we denote by

$$E(\alpha) = \int_\alpha dE_s$$

the "spectral measure" of α. These projectors $E(\alpha)$ commute with N (and N^*). Evidently,

$$\Sigma_i E(\sigma_i) = I, \qquad E(\sigma_i)E(\sigma_k) = \delta_{ik}E(\sigma_k). \tag{1}$$

To any bounded operator A we attach a "matrix," the elements of which are operators: $A_{ik} = E(\sigma_i)AE(\sigma_k)$. It follows that

$$\Sigma_k A_{ik} = E(\sigma_i)A, \qquad \Sigma_i A_{ik} = AE(\sigma_k), \tag{2}$$

$$\Sigma_{i,\,k} A_{ik} = A. \tag{3}$$

Using the function: $h(s) = s_i$ when $s \in \sigma_i (i = 1, 2, \ldots)$, we introduce a normal operator

$$N' = h(N) = \int h(s)dE_s = \Sigma_i s_i E(\sigma_i).$$

N' has a pure point spectrum with the eigenvalues among the numbers s_i and the corresponding spectral manifolds are the ranges of the projectors $E(\sigma_i)$. As, moreover, N' commutes with any E_s, we get

$$E(\sigma_i)N' = N'E(\sigma_i) = s_i E(\sigma_i). \tag{4}$$

In order to estimate how well N^1 approximates N, we observe that $|s - h(s)| \leq 1/2 s\sqrt{2}$ (= the semidiagonal of each square). Hence the operator

$$N'' = \int \{s - h(s)\}dE_s$$

is bounded and

$$\|N''f\| \leq 1/2 s\sqrt{2}\|f\|, \qquad (f \in \mathfrak{H}). \tag{5}$$

Thus we have (see, e.g., Nagy,[3] p. 45, property d)

$$N' + N'' = \int h(s)dE_s + \int \{s - h(s)\}dE_s = \int s dE_s = N, \tag{6}$$

and the domains of N and N' are equal, say $\mathfrak{D}$.

Now, let f denote an arbitrary element of $\mathfrak{D}$. Since $BN \subseteq NB$ even Bf belongs to $\mathfrak{D}$ and we have by assumption $BNf = NBf$. Using equation (6) and the fact that N'' is everywhere defined, we obtain

$$BN'f - N'Bf = -BN''f + N''Bf.$$

Replacing f by $E(\sigma_k)f$ which does belong to $\mathfrak{D}$ and applying $E(\sigma_i)$ on both sides of this equation, we get

$$E(\sigma_i)BN'E(\sigma_k)f - E(\sigma_i)N'BE(\sigma_k)f = -E(\sigma_i)\dot{B}N''E(\sigma_k)f +$$
$$E(\sigma_i)N''BE(\sigma_k)f,$$

i.e.,

$$(z_k - z_i)B_{ik}f = -B_{ik}N'f + N''B_{ik}f$$

by use of equation (4) and (on the right-hand side) $E(\sigma_i)N'' = N''E(\sigma_i)$. From this and relation (5) we derive an estimation which is the central point of the proof:

$$|z_k - z_i| \; |||B_{ik}f||| \leq \tfrac{1}{2}s\sqrt{2}|||B_{ik}||| \; ||f||. \tag{7}$$

(For a bounded operator A we denote by $|||A|||$ the greatest lower bound for the numbers c for which $||Af|| \leq c||f||$ for all f). By continuity, equation (7) remains true for any $f \in \mathfrak{H}$, whence for $i \neq k$

$$|||B_{ik}||| \leq \frac{s\sqrt{2}}{|z_k - z_i|} \; |||B_{ik}|||. \tag{8}$$

This inequality permits us to conclude that

$$|||B_{ik}||| = 0, \quad \text{i.e., } B_{ik} = 0, \tag{9}$$

for any i, k for which

$$|z_k - z_i| > s\sqrt{2}. \tag{10}$$

The only pairs i, k for which the condition (10) is not fulfilled are such where σ_i and σ_k are neighbors in the sense that they touch each other either at a corner or along a side. Even in this case we can, however, prove that $B_{ik} = 0$ holds if $i \neq k$. We divide each side of each square σ_i into n equal parts in order to introduce a new lattice, n times as fine as the original one. Consider now our two neighbors σ_i and σ_k, and let them, e.g., touch along a horizontal side, σ_i being just below σ_k. By the subdivision we get n^2 small squares σ_i^p($p = 1, 2, \ldots, n^2$) inside σ_i and n^2 small squares σ_k^q($q = 1, 2, \ldots, n^2$) inside σ_k. Then

$$E(\sigma_i) = \sum_{p=1}^{n^2} E(\sigma_i^p), \qquad E(\sigma_k) = \sum_{q=1}^{n^2} E(\sigma_k^q)$$

and hence

$$B_{ik} = E(\sigma_i)BE(\sigma_k) = \sum_{p,q=1}^{n^2} E(\sigma_i^p)BE(\sigma_k^q). \tag{11}$$

According to the above result (9), but now applied to the new, finer lattice, those terms in equation (11) which correspond to non-neighbors in the fine lattice vanish. Thus we are allowed to consider only those terms $E(\sigma_i^p)BE(\sigma_k^q)$ where σ_i^p "touches" the large square σ_k (whereas σ_k^q may be any of the small squares inside σ_k). Denoting by ρ_n the small rectangle composed of exactly these latter σ_i^p, we may recollect the terms thus considered:

$$B_{ik} = \sum_{\sigma_i} \subseteq \rho_n, \quad i = 1,2, \ldots, n \quad E(\sigma_i{}^r)BE(\sigma_k{}^r) = E(\rho_n)BE(\sigma_k).$$

(12)

This equation being valid for any natural number n, we may pass to the limit $n \to \infty$, whereby the rectangles ρ_n decrease toward their intersection which is empty because we have considered the lattice squares as "semiclosed" in the previously indicated way. By the total additivity (or "multiplicativity") of the spectral measure $E(\alpha)$ we therefore obtain

$$\lim_{n \to \infty} E(\rho_n) = 0,$$

and hence from equation (12) the desired result $B_{ik} = \lim_{n \to \infty} E(\rho_n)BE(\sigma_k) = 0$. Thus we have proved the equation

$$B_{ik} = E(\sigma_i)BE(\sigma_k) = 0 \quad \text{for} \quad i \neq k. \tag{13}$$

In order to show that B commutes with E_s for any given complex number s, we choose the original square lattice so that the point $s = x + iy$ is a lattice point. We denote by ζ the quarterplane consisting of all points with real part $<x$ and with imaginary part $<y$. This quarterplane is a sum of squares σ_i from our lattice. Now

$$E_s = E(\zeta) = \sum_i{}' E(\sigma_i)$$

where the apostrophe denotes that the summation is to be restricted to squares $\sigma_i \subseteq \zeta$. Thus we get, remembering equation (1),

$$E_sB = \sum_i{}' E(\sigma_i)B = \sum_i{}' \sum_k E(\sigma_i)BE(\sigma_k).$$

On account of equation (13) this gives

$$E_sB = \sum_i{}' E(\sigma_i)BE(\sigma_i).$$

Next, when computing BE_s in a similar manner, we obtain exactly the same result and we have thus proved that

$$BE_s = E_sB.$$

The rest of Theorem I follows easily from this relation by use of the operational calculus (see, e.g., Nagy,[3] p. 45, property b, and p. 29, top of page).

3. *On a Conversion of Theorem I.*—I. E. Segal has kindly drawn my attention to a slightly different formulation of Theorem I: *Any normal operator N has the property P that the ring of all bounded operators B commuting with N is a self-adjoint ring;* that is, whenever the ring contains B then it contains also B^*. We may ask whether this property P *characterizes* the class of normal operators. This is actually the case if we consider *bounded* operators only. For if N is a bounded operator with the above property P, then we simply choose $B = N$ and infer that $NN^* =$

N^*N, q. e. d. Next let a *non-bounded* operator T have the property P. Here we cannot choose $B = T$. This might seem to be a mere technical difficulty, but that is actually not the case as shown by the following theorem.

THEOREM II. *There exists a closed operator T (with a domain everywhere dense in $\mathfrak{H}$) which does not commute with any bounded operator, except with the numerical multiples of the identity 1.*

The operator T, constructed below as an example proving this theorem, has, furthermore, the entire complex plane as point spectrum, any complex number being a simple eigenvalue. T is, of course, not normal.

In the Hilbert space $\mathfrak{H} = \mathfrak{L}^2(-\infty < x < \infty)$ we consider the self-adjoint operators[4] $P = -id/dx$ and $Q = x\cdot$ and form the operator $T = Q + iP$, which is defined in a dense set $\mathfrak{D}$. For a function $f(x) \in \mathfrak{D}$ we have $Tf(x) = xf(x) + f'(x)$. In order first to prove that T is closed, we consider any sequence $\{f_n\}$ $(f_n \in \mathfrak{D})$ for which $\lim_n f_n (= f \in \mathfrak{H})$ and $\lim_n Tf_n (= g \in \mathfrak{H})$ exist. We then have to prove that $f \in \mathfrak{D}$ and that $Tf = g$. It is sufficient to show the existence of $\lim_n Qf_n$ and $\lim_n Pf_n$, for this implies (Q and P being closed) that $f \in \mathfrak{D}_Q \cap \mathfrak{D}_P = \mathfrak{D}$ and that $\lim_n Qf_n = Qf$, $\lim_n Pf_n = Pf$, whence $\lim_n Tf_n = \lim_n Qf_n + i \lim_n Pf_n = Qf + iPf = Tf$, q. e. d. Now, let h be an arbitrary element of $\mathfrak{D}$. Then

$$||(Q + iP)h||^2 = ||Qh||^2 + ||Ph||^2 + (Qh, iPh) + (iPh, Qh), \qquad (14)$$

$(Qh, iPh) + (iPh, Qh) = \int_{-\infty}^{\infty} xh(x)\overline{h'(x)} \, dx + \int_{-\infty}^{\infty} \overline{h'(x)}xh(x) \, dx = \int_{-\infty}^{\infty} x(d/dx)|h(x)|^2 \, dx = [x|h(x)|^2]_{-\infty}^{\infty} - \int_{-\infty}^{\infty} |h(x)|^2 \, dx \geqq 0$ $- ||h||^2$. Substituting in equation (14), we get $||Qh||^2 + ||Ph||^2 \leqq ||h||^2 + ||Th||^2$, by which inequality the convergence of $\{Qf_n\}$ and of $\{Pf_n\}$ is derived from that of $\{f_n\}$ and of $\{Tf_n\}$ putting $h = f_m - f_n$ and making $m, n \to \infty$. Thus T is a closed operator.

If s is any complex number, then the equation $Tf = sf$ may be written $f'(x) + (x - s)f(x) = 0$, the only solutions of which are $f(x) = \text{const.}$ $f_s(x)$, where

$$f_s(x) = \exp \left\{ -\frac{1}{2}(x - s)^2 \right\} \in \mathfrak{D}.$$

Thus s is a simple eigenvalue for T, the corresponding eigenelements being cf_s where c is an arbitrary complex number. Suppose now that a bounded operator B commutes with $T:BT \subseteqq TB$. In particular, $TBf_s = BTf_s$ for any s; that is $TBf_s = sBf_s$, showing that Bf_s (if $\neq 0$) is an eigenelement for T belonging to the eigenvalue s; i.e.,

$$Bf_s = c_s f_s, \qquad (15)$$

where c_s is a certain number depending on s only. On account of the regular way in which f_s depends on s, combined with the boundedness of B, we may show that c_s is a *differentiable* (i.e., analytic) function of s

in the whole s-plane, and as c_s is *bounded* (because B is bounded) we infer by Liouville's Theorem that c_s is a *constant* c. The details may be carried out in the following way. First we prove that

$$(s - s')^{-1}(f_s - f_{s'}) \to g_{s'} \qquad (\text{in } \mathfrak{H}) \text{ as } s \to s', s \neq s', \qquad (16)$$

where $g_s(x) = \partial f_s(x)/\partial s = (x - s) \exp \{ - \frac{1}{2}(x - s)^2 \}$. The relation (16) holds, of course, in the sense of pointwise convergence. Now we may show that $|f_s(x) - f_{s'}(x)| \leqq |g_{s''}(x)|/|s - s'|$ where $s'' \to s'$ as $s \to s'$. An estimation of $|g_{s''}(x)|$ justifies the application of Lebesgue's convergence theorem whereby relation (16) is proved. An immediate consequence of this result (16) is that the complex function (f_s, h) of s is differentiable at any s; h being any fixed element of $\mathfrak{H}$. In order to prove that c_s is differentiable at any given point s' we use equation (15) to write

$$c_s(f_s, f_{s'}) = (c_s f_s, f_{s'}) = (B f_s, f_{s'}) = (f_s, B^* f_{s'}).$$

Here $(f_s, f_{s'})$ and $(f_s, B^* f_{s'})$ are both differentiable (choose $h = f_{s'}$ and $= B^* f_{s'}$, respectively). Hence $c_s = (f_s, B^* f_{s'})/(f_s, f_{s'})$ is differentiable at any point s where $(f_s, f_{s'}) \neq 0$, in particular at $s = s'$, q. e. d.

From $B f_s = c f_s$ for all s we finally conclude that

$$B f = c f \qquad \text{for every } f \, \epsilon \, \mathfrak{H}, \qquad (17)$$

considering that B is a bounded (i.e., continuous) operator and that the set of all finite linear combinations of the elements f_s is *everywhere dense* in $\mathfrak{H}$. In fact this latter statement holds even by restricting s to take real values only, as we may show, e.g., by use of the Fourier integral calculus. Suppose that an element $h \, \epsilon \, \mathfrak{H}$ is orthogonal to every f_s (s real):

$$(h, f_s) = \int_{-\infty}^{\infty} h(x) \exp \{ - \frac{1}{2}(s - x)^2 \} dx = 0 \text{ for all } s. \qquad (18)$$

This means that the convolution $h(x) * \exp (- \frac{1}{2} x^2)$ of the two $\mathfrak{L}^2$-functions $h(x)$ and $\exp (- \frac{1}{2} x^2)$ is identically zero. But this implies that the product of the Fourier transforms of $h(x)$ and of $\exp (-\frac{1}{2} x^2)$ vanishes. As the latter Fourier transform is $\exp (- \frac{1}{2} x^2) \neq 0$ we infer that $h(x) = 0$. This means, however, that the set $\{f_s\}$ spans $\mathfrak{H}$. We have now established all the properties of T announced in Theorem II and the succeeding remarks.

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[1] The expression *bounded* operator means in this note a bounded, linear operator which is defined in the entire Hilbert space.

[2] *Portugaliae Math.*, 3, 1–62 (1942), particularly appendix 3, pp. 60–61.

[3] Nagy, Béla Sz., "Spektraldarstellung linearer Transformationen des Hilbertschen Raumes," *Ergeb. d. Math.*, V, 5, Berlin, 1942.

[4] Stone, M. H., *Linear Transformations in Hilbert Space*, Am. Math. Soc. Coll. Publ. XV, New York, 1932. About the self-adjoint operator $-id/dx$, see Theorem 10.9.

ON THE 3-TYPE OF A COMPLEX

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1. Introduction.—The standard algebraic invariants of a topological space depend only on the homotopy type of the space. This note will deal with part of the converse problem of the determination of the homotopy type by algebraic invariants, and will show in effect that the only one- and two-dimensional invariants which enter are the fundamental group π_1, the second homotopy group π_2, and a certain three-dimensional cohomology class of π_1 in π_2.

As in CH I,[2] we consider connected cell complexes[1] K, and denote by K^n the n-dimensional skeleton of K. We recall that the complexes K and K' are said to be of the same *n-type* if, and only if, there are maps $\phi: K_n \to K'_n$, $\phi': K'_n \to K^n$, and homotopies

$$\phi'\phi|K^{n-1} \simeq i: K^{n-1} \to K^n,$$
$$\phi\phi'|K'^{n-1} \simeq i': K'^{n-1} \to K'^n,$$

where i, i' are the identical maps. In this case we write $\phi: K^n \equiv_{n-1} K'^n$, and we assume that $n > 1$, since any two (connected) complexes are of the same 1-type. Then $\phi: K_n \equiv_{n-1} K'^n$ if, and only if,

$$\phi_r: \pi_r(K^n) \cong \pi_r(K'^n) \qquad (r = 1, \ldots, n-1), \tag{1.1}$$

according to Theorem 2 in CHI, where ϕ_r is the homomorphism induced by ϕ. The classification of complexes according to their 2-type is equivalent, under the correspondence $K \to \pi_1(K)$, to the classification of groups by the relation of isomorphism. The purpose of this note is to define an algebraic equivalent of the 3-type. Since the n-type of K depends only on K^n, we may always replace K by K^3 when we discuss the 3-type. Therefore we assume that any given complex is at most 3-dimensional.

By an *algebraic 3-type* we mean a triple, $T = (\pi_1, \pi_2, \mathbf{k})$, which consists of

(a) an arbitrary (multiplicative) group π_1,

(b) an additive, Abelian group π_2, which admits π_1 as a group of operators,

(c) a 3-dimensional cohomology class $\mathbf{k} \in H^3(\pi_1, \pi_2)$.

The algebraic 3-type of a complex K is the triple $(\pi_1(K), \pi_2(K), \mathbf{k}(K))$ consisting of the fundamental group, the second homotopy group of K (with the usual operators of $\pi_1(K)$ on $\pi_2(K)$), and the "obstruction" invariant $\mathbf{k}^3 = \mathbf{k}(K)$, defined as in CT III[4] (cf. §2 below).

Let $T = (\pi_1, \pi_2, \mathbf{k})$ and $T' = (\pi_1', \pi_2', \mathbf{k}')$ be any algebraic 3-types. By a *homomorphism*

$$\theta = (\theta_1, \theta_2): T \to T'$$

we mean a pair of homomorphisms

$$\theta_1: \pi_1 \to \pi_1', \qquad \theta_2: \pi_2 \to \pi_2' \qquad \cdot \qquad (1.2)$$

such that

$$\theta_2(xa) = (\theta_1 x)\theta_2 a, \qquad x \in \pi_1, \ a \in \pi_2, \qquad (1.3a)$$

$$\theta_2 k(x, y, z) \sim k'(\theta_1 x, \theta_1 y, \theta_1 z), \qquad x, y, z \in \pi_1; \qquad (1.3b)$$

where k and k' are (non-homogeneous) cocycles in the classes $\mathbf{k}$, $\mathbf{k}'$, respectively. The homomorphism θ is an isomorphism if, and only if, both θ_1 and θ_2 are isomorphisms; the resulting relation $T \cong T'$ of isomorphism between 3-types is clearly an equivalence relation.

We shall say that a given algebraic 3-type, T, is *realized* by a complex, K, if, and only if, $T \cong T(K)$. Let $K = K^3$ and $K' = K'^3$ be given complexes and $\phi: K \to K'$ a given map. Let $\pi_n = \pi_n(K)$, $\pi_n' = \pi_n(K')$. Then (1.3a) is satisfied by the homomorphisms ϕ_1, ϕ_2, which are induced by ϕ. It follows from the definition of $\mathbf{k}^3$ that (1.3b) is also satisfied. Therefore $\phi: K \to K'$ induces a homomorphism $\phi: T(K) \to T(K')$. If the latter is given, then a map, $K \to K'$, which induces it, will be called a (geometrical) *realization* of $\phi: T(K) \to T(K')$.

The main results are:

THEOREM 1. *Two complexes K and K' are of the same 3-type[5] if, and only if, $T(K) \cong T(K')$.*

THEOREM 2. *Any algebraic 3-type can be realized by some complex.*

THEOREM 3. *For complexes K and K' a given homomorphism $T(K) \to T(K')$ has a geometrical realization, $\phi: K \to K'$, provided that dim $K \leq 3$.*

Theorem 1 may be deduced from Theorem 3 and the statement containing equation (1.1).

2. Crossed Sequences.—The algebraic constructions relating to 3-types involve certain types of "operator sequences" of groups and homomorphisms. In general, such a sequence consists of additive groups A and B (B abelian, but not necessarily A) which admit the multiplicative groups P and Q, respectively, as groups of left operators, together with the homomorphisms

$$0 \to B \overset{\lambda}{\to} A \overset{\mu}{\to} P \overset{\nu}{\to} Q \to 1, \qquad (2.1)$$

such that $\mu\lambda(B) = 1$, $\nu\mu(A) = 1$, $\nu(P) = Q$. The homomorphisms λ, μ must be operator homomorphisms, in that

$$\lambda[(\nu p)\cdot b] = p(\lambda b), \qquad p \in P, \ b \in B; \qquad (2.2)$$

$$\mu(pa) = p(\mu a)p^{-1}, \qquad p \in P, \ a \in A. \qquad (2.3)$$

Finally,

$$a + a' - a = (\mu a)a', \qquad a, a' \in A. \tag{2.4}$$

The middle section $\mu: A \to P$, since it is subject to the conditions (2.3) and (2.4), defines A as a crossed (P, μ) module in the sense of CH II. It follows from equation (2.4), first with $a \in \mu^{-1}(1)$ and then $a' \in \mu^{-1}(1)$ that $\mu^{-1}(1)$ is in the center of A and that μA operates simply on $\mu^{-1}(1)$.

We need only two special types of such operator sequences.

Crossed sequences are operator sequences (2.1) which are exact sequences. The crossed (P, μ) module A of such a sequence determines the crossed sequence up to isomorphism, with $Q \cong P/\mu A$, $B \cong \mu^{-1}(1) \subset A$, and with the operators of Q on B determined by equation (2.2). Hence the theory of crossed modules is equivalent to that of crossed sequences, as developed[6] in CT III.

Each crossed sequence determines an algebraic 3-type in the following way. For each $q \in Q$ select a representative $u(q) \in \nu^{-1}q$ in P, with $u(1) = 1$. Then $u(q)u(q')u(qq')^{-1} = f(q, q')$ lies in $\nu^{-1}(1) = \mu(A)$. Select $a(q, q')$ $\in \mu^{-1}f(q, q')$ with $a(q, 1) = a(1, q') = 0$. Then, for $q, r, s \in Q$,

$$\delta a(q, r, s) = u(q) \cdot a(r, s) + a(q, rs) - a(qr, s) - a(q, r)$$

lies in $\mu^{-1}(1)$. The function k with $k(q, r, s) = \lambda^{-1}[\delta a(q, r, s)]$ is then defined, and is a (non-homogeneous) 3-dimensional cocycle of Q in B. Its cohomology class, $\mathbf{k}$, which is independent[7] of the choice of u and a, is called the *obstruction* of the sequence, and the triple $(Q, B, \mathbf{k})$ is the (unique) algebraic 3-type associated with the sequence.

Homotopy Systems of dimension 3, as defined in CH II, are operator sequences (2.1) in which P is a free group, A a free crossed (P, μ) module, and B a free (abelian) Q-module, and in which $\nu^{-1} 1 = \mu A$ (exactness at P). Since $Q \cong P/\mu(A)$, Q need not be given in advance.

Each homotopy system (2.1) determines a certain crossed sequence, as follows. Since $\mu\lambda(B) = 1$, μ induces a homomorphism $\mu': A/\lambda B \to P$. By equation (2.2), the given operators of P on A induce operators of P on $A/\lambda B$. By equation (2.3) $\mu^{-1}(1) \subset A$ is closed under operation by P; since μA operates simply on $\lambda B \subset \mu^{-1}(1)$, operators of $Q \cong P/\mu A$ on $\mu^{-1}(1)/\lambda B$ are induced. Using the identity injection λ', we thus have a crossed sequence

$$0 \to \mu^{-1}(1)/\lambda B \overset{\lambda'}{\to} A/\lambda B \overset{\mu'}{\to} P \overset{\nu'}{\to} Q \to 1. \tag{2.5}$$

We call this the sequence *derived* from the homotopy sequence (2.1).

The geometric applications are as follows. If K is a complex, the sequence of homotopy groups

$$0 \to \pi_2(K) \to \pi_2(K, K^1) \to \pi_1(K^1) \to \pi_1(K) \to 1, \tag{2.6}$$

with the usual mappings and operators, is a crossed sequence.   We define $k(K)$ as the obstruction of this sequence.   This agrees with the geometric definition[8] of this invariant, as may be proved using one of the known additivity theorems[9] for relative homotopy groups.

The homotopy system (CH II) of the 3-dimensional cell complex K consists of the homotopy groups $\pi_1(K)$, $\rho_1 = \pi_1(K^1)$ and $\rho_n = \pi_n(K^n, K^{n-1})$ for $n = 2, 3$, together with the usual operators, and the homomorphisms

$$0 \to \rho_3 \xrightarrow{d_3} \rho_2 \xrightarrow{d_2} \rho_1 \xrightarrow{d_1} \pi_1 \to 1, \tag{2.7}$$

where d_1 is the injection homomorphism and $d_n = j_{n-1}\beta_n$, for $n = 2, 3$, is the composite of the boundary and injection homomorphisms

$$\rho_n \xrightarrow{\beta_n} \pi_{n-1 (K_{n-1})} \xrightarrow{j_{n-1}} \rho_{n-1}.$$

The derived sequence

$$0 \to d_2^{-1}(1)/d_3\rho_3 \to \rho_2/d_3\rho_3 \to \rho_1 \to \pi_1 \to 1 \tag{2.8}$$

of this homotopy sequence is isomorphic to the crossed sequence (2.6) of K; in other words $\pi_2(K) \cong d_2^{-1}(1)/d_3\rho_3$ and $\pi_3(K, K^1) \cong \rho_3/d_3\rho_3$, with the mappings and operators corresponding under the isomorphisms. Indeed, the first isomorphism follows from the exactness of the homotopy sequences for the pairs K^2, K^1 and K^3, K^2, together with $\pi_2(K^1) = 0$, $\pi_3(K^3, K^2) = 0$, while the second isomorphism follows from the known exactness of the homotopy sequence

$$\pi_3(K^3, K^2) \to \pi_3(K^3, K^1) \to \pi_3(K^3, K^1)$$

for the triple K^3, K^2, K^1.

3. Realization of an Algebraic 3-Type.—Each homotopy system determines a derived crossed sequence and thence an algebraic 3-type. Theorem 2 will be proved by reversing this process.   Let (π_1, π_2, k) be any algebraic 3-type.   The group π_1 can be represented as the image of a free group X under a homomorphism ν.   By Theorem C in CT III we can construct[10] a crossed sequence

$$0 \to \pi_2 \xrightarrow{\lambda} A \xrightarrow{\mu} X \xrightarrow{\nu} \pi_1 \to 1 \tag{3.1}$$

which realizes the given algebraic 3-type.   To construct a corresponding homotopy system with $\rho_1 = X$, take ρ_2, as in §2 of CH II, to be the free crossed (X, d_2)-module with symbolic generators (x, a) for all $x \in X$ and all a in any chosen set of generators of A and with $d_2: \rho_2 \to X$ determined by $d_2(x, a) = x(\mu a)x^{-1}$.   By Lemma 2 in CH II an operator homomorphism $\omega: \rho_2 \to A$ onto A is determined by setting $\omega(1, a) = a$.   Then $d_2 = \mu\omega$, and, since ω and d_2 are operator homomorphisms, the abelian sub-

groups $\omega^{-1}(0) \subset d_1^{-1}(1)$ of ρ_1 are invariant under the operators of ρ_1, and the crossed sequence (3.1) is isomorphic (under ω) to the crossed sequence

$$0 \to d_2^{-1}(1)/\omega^{-1}(0) \to \rho_2/\omega^{-1}(0) \to \rho_1 \to \pi_1 \to 1. \quad (3.2)$$

The abelian group $\omega^{-1}(0)$ admits π_1 as a group of operators according to the rule $(vx)b = xb$ for $x \in X$ and $b \in \omega^{-1}(0)$. Hence there is a free π_1-module ρ_3 with an operator homomorphism $d_3: \rho_3 \to \omega^{-1}(0) \subset \rho_2$ onto $\omega^{-1}(0)$. We have thus constructed a homotopy system

$$0 \to \rho_3 \overset{d_3}{\to} \rho_2 \overset{d_2}{\to} \rho_1 \overset{v}{\to} \pi_1 \to 1 \quad (3.3)$$

for which the derived crossed sequence (3.2) is isomorphic to (3.1).

Theorem 2 of CH II asserts that the homotopy system (3.3) can be realized as the homotopy system of a 3-dimensional complex K. The derived sequence (3.2) is then on the one Hand isomorphic to the relative homotopy sequence (2.6) of the complex K, with obstruction the obstruction of the space K, and on the other hand to the given sequence (3.1) with the preassigned obstruction k. Hence K realizes the given 3-type, as asserted in Theorem 2.

4. Mappings of Complexes with Operators.—An abstract closure finite cell complex C—that is, a system of free abelian groups C_q and homomorphisms

$$C_0 \overset{\partial}{\leftarrow} C_1 \overset{\partial}{\leftarrow} C_2 \overset{\partial}{\leftarrow} \dots$$

with $\partial\partial = 0$—has free operators in the multiplicative group W if each C_q is a free W-module and each $w \in W$ a chain transformation ($w\partial = \partial w$). Select a preferred W-base for C_0, consisting of certain 0-cells, one of which we call the *special* 0-cell, and define the homomorphism J of C_0 into the group of integers by setting $J(wc_0) = 1$ for each preferred 0-cell c_0. We require that C_0 be augmentable, as[11] in HSO II, p. 54; i.e., that $C_0 \neq 0$ and $J\partial = 0$. Under these conditions we call (W, C) a *complex with free operators*.

A homomorphism (f_0, λ) of one such complex (W, C) into a second (W', C') consists of homomorphisms $f_0: W \to W'$, $\lambda_q: C_q \to C_q'$, such that λ is a chain transformation ($\lambda\partial = \partial\lambda$), $\lambda w = (f_0 w)\lambda$ for each $w \in W$, and $\lambda_0 c_0$ is the special 0-cell of C' whenever c_0 is in the preferred W-base of C. Then $J\lambda_0 = J$, and λ is also augmentable, in the sense of HSO II.

Any multiplicative group W determines such a complex K_W, as in HSO II, with q-cells $(w_0, \dots, w_q)$ for $w_i \in W$ and the preferred W-base for C_0 consisting of the special 0-cell (1). Any homomorphism $f_0: W \to W'$ induces a homomorphism $(f_0, f): K_W \to K_{W'}$ with $f_q: C_q(K_W) \to C_q(K_{W'})$ determined by the formula

$$f_q(w_0, \ldots, w_q) = (f_0 w_0, \ldots, f_0 w_q), \qquad w_i \, \epsilon \, W.$$

For any complex K the abstract cell complex $C(\check{K})$, consisting of the chain groups $C_n = H_n(\check{K}^n, \check{K}^{n-1})$ of the universal covering complex[12] $\check{K}$ is a complex with free operators in the group $W \cong \pi_1(K)$ of covering transformations. For the preferred W-base of C_0 select *a* 0-cell over each 0-cell of K, and as the special 0-cell select the 0-cell carried by the base point of $\check{K}$. A homomorphism $(f_0, \lambda):C(\check{K}) \rightarrow C(\check{K}')$ is then a chain mapping in the sense of CH II, §9, and in particular $\lambda_0:C_0(\check{K}) \rightarrow C_0(\check{K}')$ can be realized geometrically by a map $K^0 \rightarrow e'^0$, where e'^0 is the base point in K' (of course all of $\check{K}^0$ need not map into the base point in $\check{K}'$).

If the complexes (W, C) and (W', C') are acyclic in dimensions less than q, their integral homology groups $H = H_q(C)$ and $H' = H_q(C')$ in this dimension have operators in W and W', respectively. C (and likewise C') then has an obstruction cohomology class $1 \, \epsilon \, H^{q+1}(W, H)$, determined as in HSO II, Theorem 5.1, as the obstruction of any homomorphism of the q-dimensional skeleton of K_W into C. For $q = 2$, the system $(W, H, 1)$ determined by C is an algebraic 3-type. For any q, a *homomorphism*

$$(f_0, h): (W, H, 1) \rightarrow (W', H', 1') \tag{4.1}$$

will mean a pair of homomorphisms $f_0: W \rightarrow W'$ and $h:H \rightarrow H'$ which satisfy conditions analogous to equation (1.3).

If C^{q+1} is the $(q + 1)$-dimensional skeleton of C, any homomorphism $(f_0, \lambda): C^{q+1} \rightarrow C'^{q+1}$ induces a homomorphism $h:H \rightarrow H'$. By the argument of Theorem 5.1 in HSO II, f_0 and h satisfy the analogue of (1.3b); they obviously satisfy (1.3a). Therefore (f_0, h) is a homomorphism of the form (4.1). We then call (f_0, λ) a *combinatorial realization* of (f_0, h).

THEOREM 4. *For complexes C, C' with free operators in W, W', acyclic in dimensions less than $q(q > 0)$, any homomorphism (4.1) has a combinatorial realization $(f_0, \lambda): C^{q+1} \rightarrow C'^{q+1}$.*

This theorem is an extension of part of Theorem 7.1 in HSO II, and is established by the argument there (pp. 62, 63) with the following modifications. If 1 and 1' are (homogeneous) cocycles in the classes 1 and 1', respectively, the analogue of (1.3b) shows that there is a cochain m': $C_q(K_w) \rightarrow H'$ such that $h1 = 1f_{q+1} + \delta m'$. Since $C_q(K_w)$ is a free W-module, the homomorphism m' can be lifted to an operator homomorphism $g': C_q(K_w) \rightarrow Z_q(C')$, with $\eta'g = m'$. The first equation of (7.5) becomes $\eta'F_q = h\eta E_q$, and the required realization λ is defined by

$$\lambda_i = \alpha_i'f_i\gamma_i: C_i \rightarrow C_i' \qquad i = 0, \ldots, q-1,$$
$$\lambda_q = \alpha_q'f_q\gamma_q + g'\gamma_q + F_q: C_q \rightarrow C_q'.$$

The cited calculations then show the existence of a suitable λ_{q+1}.

5. *Proof of Theorem 3.*—For a complex K^3 the associated chain system $C(\bar{K}^3)$ is acyclic in dimensions less than 2, and the obstruction $\mathbf{k}(K)$, as defined in §2 above, agrees with the obstruction 1 of $C(K^3)$. Indeed, choose $u(q) \in \rho_1$ and $a(q, r) \in \rho_2/d_2\rho_3$ as in the definition of $\mathbf{k}$ for the sequence (2.8), and let ω be the natural homomorphism $\omega: \rho_2 \to \rho_2/d_2\rho_3$. Choose $R^1(q) = u(q)$ and $R^2(q, r) \in \omega^{-1}a(q, r)$. Then $\omega\delta R^2 = \delta a$, whence $\delta R^2(q, r, s) \in \omega^{-1}\mu^{-1}(1) = d_2^{-1}(1)$. Using the operator homomorphisms h_n: $\rho_n \to C_n$ of §12 of CH II, set $f^n = h_n R^n \in C^n(\pi_1, C_n)$ for $n = 1, 2$, and $f^0 = c^0$, where c^0 is the special 0-cell in C_0. Then it may be verified that $\delta f^i = \delta f^{i+1}$ for $i = 0, 1$; hence, by Theorem 6.3 in HSO II, δf^2 is the obstruction 1 of C. By Lemma 5 in CH II, h^2 induces an isomorphism $d_2^{-1}(1)/d_2\rho_3 \cong H$. It follows that the isomorphisms $\pi_1 \cong W$, $d_2^{-1}(1)/d_2\rho_3 \cong H$ carry $\mathbf{k}(K^3)$ into the obstruction 1 of C.

Now consider two complexes K^3, K'^3 with their associated chain systems $C = C(\bar{K}^3)$ and $C' = C(\bar{K}'^3)$, acyclic in dimensions less than 2. Any homomorphism $\theta: T(K) \to T(K')$ on the algebraic 3-types of K and K' satisfies the hypotheses of Theorem 4 for $q = 2$, hence has a combinatorial realization $\lambda: C \to C'$. By Theorem 16 in CH II, λ has a geometrical realization $\phi: K \to K'$. Because of the natural isomorphisms $H \cong \pi_2(K)$, $H' = \pi_2(K')$, the map ϕ is a geometrical realization of θ, and Theorem 3 is proved.

Theorem 3 can also be proved without the use of chain groups and covering complexes by combining certain theorems of CT III, on the "deviation" of exact sequences, with theorems in CH II on the realizability of homomorphisms of homotopy systems.

6. *A Sufficiency Theorem.*—By a *sufficiency theorem* we mean one which states that certain invariants are sufficiently powerful to insure that, within a definite category, any mapping which induces isomorphisms of these invariants is an equivalence. For instance the theorem quoted in §1, which states that formula (1.1) implies $\phi: K^{n+1} \equiv_n K'^{n+1}$, is a sufficiency theorem, within any category of CW-complexes and n-homotopy classes of maps, $K^{n+1} \to K'^{n+1}$. A realizability theorem, like Theorem 3 or Theorem 4 above, is one which states that a homomorphism of some kind of algebraic invariant can be realized by a mapping of objects in the category.

Let C, C' mean the same as at the beginning of §4, let $W = W'$, and let $C_n = 0$, $C_n' = 0$ if $n > q + 1$. Let $\lambda \simeq_e \mu$ mean the same as $\lambda \simeq \mu$ (dim $\leq q$) in HSO II, where λ is equivariant. We shall write $\lambda: C \equiv_e C'$ if, and only if, there is an equivariant homomorphism $\lambda': C' \to C$, such that $\lambda'\lambda \simeq_e 1$, $\lambda\lambda' \simeq_e 1$. Then our sufficiency theorem, which is analogous to Theorem 2 in CH I, is

THEOREM 5. *If $\lambda: C \to C'$ induces isomorphisms $H_n(C) \cong H_n(C')$ for $n = 0, \ldots, q$, then $\lambda: C \equiv_e C'$.*

This follows from the arguments used in a forthcoming paper.[18] It is proved by constructing an "abstract" mapping cylinder of λ and transcribing into algebraic terms the proof of the analogous theorem on CW-complexes.

* This note arose from consultations during the tenure of a John Simon Guggenheim Memorial Fellowship by MacLane.

[1] Whitehead, J. H. C., "Combinatorial Homotopy I and II," *Bull. A.M.S.*, **55**, 214–245 and 453–496 (1949). We refer to these papers as CH I and CH II, respectively.

[2] By a complex we shall mean a connected CW complex, as defined in §5 of CH I. We do not restrict ourselves to finite complexes. A fixed 0-cell $e^0 \in K^0$ will be the base point for all the homotopy groups in K.

[4] MacLane, S., "Cohomology Theory in Abstract Groups III," *Ann. Math.*, **50**, 736–761 (1949), referred to as CT III.

[5] An (unpublished) result like Theorem 1 for the homotopy type was obtained prior to these results by J. A. Zilber.

[6] CT III uses in place of equation (2.4) the stronger hypothesis that λB contains the center of A, but all the relevant developments there apply under the weaker assumption (2.4).

[7] Eilenberg, S., and MacLane, S., "Cohomology Theory in Abstract Groups II," *Ann. Math.*, **48**, 326–341 (1947).

[8] Eilenberg, S., and MacLane, S., "Determination of the Second Homology . . . by Means of Homotopy Invariants," these PROCEEDINGS, **32**, 277–280 (1946).

[9] Blakers, A. L., "Some Relations Between Homology and Homotopy Groups," *Ann. Math.*, **49**, 428–461 (1948), §12.

[10] The hypothesis of Theorem C, requiring that $\nu^{-1}(1)$ not be cyclic, can be readily realized by suitable choice of the free group X, but this hypothesis is not needed here (cf. [6]).

[11] Eilenberg, S., and MacLane, S., "Homology of Spaces with Operators II," *Trans. A.M.S.*, **65**, 49–99 (1949); referred to as HSO II.

[12] $C(\bar{K})$ here is the $C(K)$ of CH II. Note that $\bar{K}$ exists and is a CW complex by (N) of p. 231 of CH I and that $p^{-1}K^n = \bar{K}^n$, where p is the projection $p: \bar{K} \to K$.

[13] Whitehead, J. H. C., "Simple Homotopy Types." If $W = 1$, Theorem 5 follows from (17:3) on p. 155 of S. Lefschetz, *Algebraic Topology*, (New York, 1942) and arguments in §6 of J. H. C. Whitehead, "On Simply Connected 4-Dimensional Polyhedra" (*Comm. Math. Helv.*, **22**, 48–92 (1949)). However this proof cannot be generalized to the case $W \neq 1$.

EQUILIBRIUM POINTS IN N-PERSON GAMES

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One may define a concept of an *n*-person game in which each player has a finite set of pure strategies and in which a definite set of payments to the *n* players corresponds to each *n*-tuple of pure strategies, one strategy being taken for each player. For mixed strategies, which are probability

distributions over the pure strategies, the pay-off functions are the expectations of the players, thus becoming polylinear forms in the probabilities with which the various players play their various pure strategies.

Any n-tuple of strategies, one for each player, may be regarded as a point in the product space obtained by multiplying the n strategy spaces of the players. One such n-tuple counters another if the strategy of each player in the countering n-tuple yields the highest obtainable expectation for its player against the $n - 1$ strategies of the other players in the countered n-tuple. A self-countering n-tuple is called an equilibrium point.

The correspondence of each n-tuple with its set of countering n-tuples gives a one-to-many mapping of the product space into itself. From the definition of countering we see that the set of countering points of a point is convex. By using the continuity of the pay-off functions we see that the graph of the mapping is closed. The closedness is equivalent to saying: if $P_1, P_2, \ldots$ and $Q_1, Q_2, \ldots, Q_n, \ldots$ are sequences of points in the product space where $Q_n \to Q$, $P_n \to P$ and Q_n counters P_n then Q counters P.

Since the graph is closed and since the image of each point under the mapping is convex, we infer from Kakutani's theorem[1] that the mapping has a fixed point (i.e., point contained in its image). Hence there is an equilibrium point.

In the two-person zero-sum case the "main theorem"[2] and the existence of an equilibrium point are equivalent. In this case any two equilibrium points lead to the same expectations for the players, but this need not occur in general.

[1] Kakutani, S., *Duke Math. J.*, 8, 457–459 (1941).

[2] Von Neumann, J., and Morgenstern, O., *The Theory of Games and Economic Behaviour*, Chap. 3, Princeton University Press, Princeton, 1947.

REMARK ON WEYL'S NOTE "INEQUALITIES BETWEEN THE TWO KINDS OF EIGENVALUES OF A LINEAR TRANSFORMATION"*

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Communicated by H. Weyl, November 25, 1949

In the note quoted above H. Weyl proved a Theorem involving a function $\varphi(\lambda)$ and concerning the eigenvalues α_i of a linear transformation A and those, κ_i, of A^*A. If the κ_i and $\lambda_i = |\alpha_i|^2$ are arranged in descending order,

$$\kappa_1 \geq \kappa_2 \geq \ldots \geq \kappa_n \ (\geq 0) \text{ and } \lambda_1 \geq \lambda_2 \geq \ldots \geq \lambda_n,$$

they satisfy the inequalities

$$\lambda_1 \leq \kappa_1, \ \lambda_1\lambda_2 \leq \kappa_1\kappa_2, \ \ldots, \lambda_1 \ldots \lambda_n \leq \kappa_1 \ldots \kappa_n$$

(*loc. cit.*, equation 4). The theorem follows from them by applying to $a_i = \log \kappa_i$, $b_i = \log \lambda_i$ the following:

LEMMA. *Given two sequences of real numbers a_1, ..., a_m and b_1, ..., b_m such that*[1]

$$b_1 \geq b_2 \geq \ldots \geq b_m$$

$$b_1 + \ldots + b_q \leq a_1 + \ldots + a_q \quad \text{(for } q = 1, \ldots, m), \quad (1)$$

the inequality

$$\omega(b_1) + \ldots + \omega(b_m) \leq \omega(a_1) + \ldots + \omega(a_m) \quad (2)$$

holds for any convex increasing function $\omega(x)$.

According to this lemma the condition to be imposed upon the function $\varphi(\lambda)$ of the Theorem is that $\varphi(e^\xi)$ be a convex and increasing function of ξ. (Weyl's accessory condition $\varphi(0) = \lim_{\lambda \to 0} \varphi(\lambda) = 0$ proves superfluous.) In a joint note by G. H. Hardy, J. E. Littlewood and myself,[2] a statement S somewhat similar to this lemma was given, and rediscovered a few years later by J. Karamata.[3] It differs from the lemma in two points: [1] The last of the inequalities (1) is replaced by the corresponding equation

$$b_1 + \ldots + b_m = a_1 + \ldots + a_m;$$

[2] the function $\omega(x)$ is assumed to be convex but need not be increasing. I wish to point out that there is a very simple way for deducing the lemma from this statement S.

Indeed under the assumptions of the lemma, set

$$(a_1 + \ldots + a_m) - (b_1 + \ldots + b_m) = c \geq 0$$

and add one further term $b_{m+1} \leq b_m$ to the sequence b. With $a_{m+1} = b_{m+1} - c$ statement S becomes applicable to the two sequences a, b of length $m + 1$:

$$\omega(b_1) + \ldots + \omega(b_{m+1}) \leq \omega(a_1) + \ldots + \omega(a_{m+1}). \quad (3)$$

But since $\omega(x)$ is supposed to be increasing and $a_{m+1} \leq b_{m+1}$ we have

$$\omega(a_1) + \ldots + \omega(a_m) + \omega(a_{m+1}) \leq \omega(a_1) + \ldots + \omega(a_m) + \omega(b_{m+1}). \quad (4)$$

Inequalities (3) and (4) give inequality (2).

* These PROCEEDINGS, **35**, 408–411 (1949).

[1] For reasons that will presently become clear, it is wiser not to require $a_1 \geq a_2 \geq \ldots \geq a_m$. The inequalities, equation (1), once they hold, are not destroyed if afterwards the a_i are re-arranged in descending order.

[2] Hardy, G. H., Littlewood, J. E., and Polya, G., "Some Simple Inequalities Satisfied by Convex Functions," *Messenger Math.*, **58**, 145–152 (1929); cf., the book *Inequalities*, by the same authors, Cambridge, 1934, p. 89.

[3] Karamata, J., "Sur une inégalité relative aux fonctions convexes," *Pub. Math. Univ. Belgrade*, **1**, 145–148 (1932).

SINGULAR POINTS OF FUNCTIONS DEFINED BY C-FRACTIONS

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A C-fraction is a continued fraction of the form

$$1 + \underset{n=1}{\overset{\infty}{K}} \left(\frac{d_n x^{\alpha_n}}{1} \right), \tag{1}$$

where the exponents α_n are positive integers and where $d_n \neq 0$ for all $n \geq 1$. Let $A_n(x)/B_n(x)$ be the nth approximant of the C-fraction (1) and set

$$h_n = \sum_{k=1}^{n+1} \alpha_k.$$

To every C-fraction corresponds a power series (see Leighton and Scott[1])

$$P(x) = \sum_{k=0}^{\infty} c_k x^k, \quad c_0 = 1, \tag{2}$$

in such a way that

$$P(x) - \frac{A_n(x)}{B_n(x)} = (-1)^n \left(\prod_{k=1}^{n+1} d_k \right) x^{h_n} + \ldots.$$

Note that this is only a formal identity; it is not, in general, assumed that $P(x)$ is convergent.

Applying Worpitzky's criterion (see reference 3, p. 42) one shows easily that the C-fraction (1) converges to a meromorphic function for $|x| < 1$ provided

$$\alpha_n \geq \alpha_{n-1} \text{ for all } n \geq 2, \quad \lim_{n \to \infty} \alpha_n = \infty, \quad \lim_{n \to \infty} |d_n|^{1/\alpha_n} = 1. \tag{3}$$

It has been conjectured by Leighton that all C-fractions satisfying the conditions (3) represent functions which have the unit circle as a natural boundary. Scott and Wall[2] proved this conjecture for the case $d_n = d$, $\alpha_n = m^{n-1}$ where either d is real and m is odd or d is negative and m is an arbitrary integer.

The purpose of this note is to prove the following two theorems:

THEOREM 1. *If the C-fraction (1) satisfies conditions (3) then the mero-morphic function $f(x)$ to which it converges for $|x| < 1$ has at least one singular point, which is not a pole, on the circle $|x| = 1$.*

THEOREM 2. *If the C-fraction (1) satisfies conditions (3) and if in addition there exists a sequence of positive integers $\{\mu_k\}$ with $\lim \mu_k \neq \infty$ which has the property that for every k there exists an $n(k)$ such that μ_k divides α_n for all $n > n(k)$ then the function $f(x)$ to which the C-fraction converges for $|x| < 1$ has the circle $|x| = 1$ as a natural boundary.*

Theorem 2, which contains the results of Scott and Wall as a special case, is derived from Theorem 1 by showing that if $f(x)$ has a singular point at $x = e^{i\alpha}$ it also has singular points at

$$x = e^{i(\alpha + 2\pi n/\mu_k)}$$

for all n and all k.

To prove Theorem 1 we begin with some preliminary observations. Let k_n and m_n denote the numbers of terms, before terms involving equal powers of x have been grouped together, of $A_n(x)$ and $B_n(x)$, respectively. Since $k_{n+1} = k_n + k_{n-1}$, $k_0 = 1$, $k_1 = 2$; $m_{n+1} = m_n + m_{n-1}$, $m_1 = 1$, $m_1 = 2$ it follows that

$$k_n \leqq 2^n, \quad m_n \leqq 2^{n-1} \text{ for all } n \geqq 1.$$

Let s_n and t_n be the degrees of the polynomials $A_n(x)$ and $B_n(x)$, respectively. If in the C-fraction (1) $\alpha_n \geqq \alpha_{n-1}$ for all $n \geqq 2$ then

$$s_{2n} = \sum_{k=1}^{n} \alpha_{2k}, \quad s_{2n+1} = \sum_{k=0}^{n} \alpha_{2k+1},$$

$$t_{2n} = \sum_{k=1}^{n} \alpha_{2k}, \quad t_{2n+1} = \sum_{k=1}^{n} \alpha_{2k+1}.$$

so that $s_n \geqq t_n$ for all $n \geqq 1$. Further $h_n - s_n \geqq s_n$ and hence

$$2(h_n - s_n) \geqq h_n. \tag{4}$$

If in addition $\lim \alpha_n = \infty$ then

$$\lim_{n \to \infty} \frac{h_{2n} - s_{2n}}{2n+1} = \lim_{n \to \infty} \left(\frac{n+1}{2n+1}\right) \frac{\alpha_1 + \ldots + \alpha_{2n+1}}{(n+1)} = \infty,$$

and similarly for $\lim(h_{2n+1} - s_{2n+1})/(2n+2)$, so that

$$\lim_{n \to \infty} \frac{h_n - s_n}{n+1} = \infty.$$ (5)

Now let $f(x)$ be the function to which the C-fraction (1), which from here on is assumed to satisfy the conditions (3), converges and assume that $f(x)$ is meromorphic for all $|x| < 1 + \epsilon, \epsilon > 0$. Let $2\eta = \epsilon$ then n_0 can be found such that

$$|d_n|^{1/a_n} > (1 + \eta)^{-1/4} \text{ for } n \geq n_0.$$

The C-fraction

$$1 + \underset{n = n_0}{\overset{\infty}{K}} \left(\frac{d_n x^{a_n}}{1}\right)$$ (6)

converges to a function $f_{n_0}(x)$ which is meromorphic for $|x| < 1 + \epsilon$. Set

$$d_n' = d_{n_0+n-1}, \qquad a_n' = a_{n_0+n-1}$$

then equation (6) becomes

$$1 + \underset{n = 1}{\overset{\infty}{K}} \left(\frac{d_n' x a_n'}{1}\right).$$ (7)

The quantities $A_n'(x)$, $B_n'(x)$, $P'(x)$, s_n', t_n' and h_n' will now be understood to have the same meaning for equation (7) as the corresponding unprimed quantities have for equation (1).

Since $f_{n_0}(x)$ is meromorphic for $|x| < 1 + \epsilon$ it can be written as

$$f_{n_0}(x) = a(x)/b(x)$$

where $a(x)$ and $b(x)$ are holomorphic for $|x| < 1 + \epsilon$ and where

$$a(x) = \sum_{k=0}^{\infty} a_k x^k, a_0 = 1, \qquad b(x) = \sum_{k=0}^{\infty} b_k x^k, b_0 = 1.$$

It is easily verified that there exists a $\delta > 0$ such that $f_{n_0}(x)$ is holomorphic for $|x| < \delta$. Thus the power series expansion of $f_{n_0}(x)$ is $P'(x)$ (see reference 1, Theorem 1.5) and the following power series identity is valid

$$(-1)^n \left(\overset{n+1}{\underset{k=1}{\Pi}} d_k'\right) x^{h'_n} + \ldots = P'(x) - \frac{A_n'(x)}{B_n'(x)} = f_{n_0}(x) - \frac{A_n'(x)}{B_n'(x)} =$$

$$\frac{a(x)B_n'(x) - b(x)A_n'(x)}{b(x)B_n'(x)}.$$

Since $B_n'(0) = 1 = b(0)$ it follows from the above that

$$a(x)B_n'(x) - b(x)A_n'(x) = (-1)^n \left(\overset{n+1}{\underset{k=1}{\Pi}} d_k'\right) x^{h_n'} + \ldots. \quad (8)$$

Denote by u_n the coefficient of $x^{h'_n}$ in the power series expansion of

$$(a(x)B_n{}'(x) \,-\, b(x)A_n{}'(x))/ \prod_{\substack{h\,=\,1 \\ |d_k'|\,>\,1}}^{n\,+\,1}{}' \; d_k'.$$

Then

$$|u_n| \,<\, (k_n + m_n) \; \max. \; (|a_k|, |b_k|)$$
$$h_n{}' - \max. \; (s_n{}', t_n{}') \leq k \leq h_n{}'$$

Since max. $(s_n{}', t_n{}') = s_n{}'$ and since $k_n \leq 2^n$, $m_n \leq 2^{n-1}$ one can write

$$|u_n| \,<\, 2^{n+1} \; \max. \; (|a_k|, |b_k|).$$
$$h_n{}' - s_n{}' \leq k \leq h_n{}'$$

Now choose n_1 in such a way that

$$|a_n| \,<\, (1 + \eta)^{-n}, \; |b_n| \,<\, (1 + \eta)^{-n}, \; \text{for } n > n_1/2.$$

Then, since $h_n{}' - s_n{}' \geq h_n{}'/2 > n/2$,

$$|u_n| \,<\, 2^{n+1}(1 + \eta)^{-(h_n{}'-s_n{}')} \text{ for } n > n_1.$$

From relation (8) follows that

$$|u_n| \,>\, (1 + \eta)^{-h_n{}''/4}.$$

The following inequality is therefore valid

$$2^{n+1} \,>\, (1 + \eta)^{h_n{}'-s_n{}'-h_n{}''/4}.$$

Thus in view of relation (4)

$$2^{n+1} \,>\, (1 + \eta)^{(h_n{}'-s_n{}')/2},$$

and finally

$$\frac{h_n{}' - s_n{}'}{n + 1} \,<\, 2 \log_{1+\eta} 2,$$

which contradicts relation (5) regardless of how small η is. This completes the proof of Theorem 1.

[1] Leighton, W., and Scott, W. T., "A General Continued Fraction Expansion," *Bull. Am. Math. Soc.*, **45**, 596–605 (1939).

[2] Scott, W. T., and Wall, H. S., "Continued Fraction Expansions for Arbitrary Power Series," *Ann. Math.* (2), **41**, 328–340 (1940).

[3] Wall, H. S., "Analytic Theory of Continued Fractions," New York, 1948

THE SECONDARY BOUNDARY OPERATOR

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1. The Sequence $S(K)$. Let $H_n = H_n(K)$ be the nth homology group of a complex[1] K and let

$$\Pi_n = \Pi_n(K) = \pi_n(K) \qquad \Gamma_n = \Gamma_n(K) = i_n \pi_n(K^{n-1}),$$

where $i_n : \pi_n(K^{n-1}) \to \pi_n(K^n)$ is the injection ($n \geq 2$). Then a sequence of homomorphisms

$$S(K): \quad \ldots \to H_{n+1} \xrightarrow{\mathfrak{b}} \Gamma_n \xrightarrow{\mathfrak{i}} \Pi_n \xrightarrow{\mathfrak{j}} H_n \xrightarrow{\mathfrak{b}} \Gamma_{n-1} \xrightarrow{\mathfrak{i}} \ldots,$$

terminating with $H_3 \to 0 \to \Pi_2 \to H_2 \to 0$, is defined as follows. $\mathfrak{j}_n$ is the natural homomorphism and $\mathfrak{i}_n = i_n' | \Gamma_n$, where $i_n' : \pi_n(K^n) \to \Pi_n$ is the injection. We assume that H_n is defined as

$$H_n = Z_n - d_{n+1} C_{n+1} \qquad (n \geq 3),$$

where $C_n = \pi_n(K^n, K^{n-1})$, $Z_n = d_n^{-1}(0) \subset C_n$ and $d_{n+1} : C_{n+1} \to C_n$ is the resultant of the boundary homomorphism, $\beta_{n+1} : C_{n+1} \to \pi_n(K^n)$, followed by the injection $j_n : \pi_n(K^n) \to C_n$. Let $z \in Z_{n+1}$. Since $j_n \beta_{n+1} z = 0$ it follows from the exactness of the homotopy sequence of K^{n-1}, K^n that $\beta_{n+1} z \in \Gamma_n$. Also $\beta_{n+1} d_{n+2} = 0$, since $\beta_{n+1} j_{n+1} = 0$. Therefore $\beta_{n+1} | Z_{n+1}$ induces a homomorphism, $\mathfrak{b}_{n+1} : H_{n+1} \to \Gamma_n$, which is the one in $S(K)$.

THEOREM 1. *The sequence $S(K)$ is exact.*[2]

Let $m \geq 4$ and let

$$S_m(K): \quad \Pi_m \to \Gamma_{m-1} \to \ldots$$

be the part of $S(K)$ which begins with Π_m. We write $S_\infty(K) = S(K)$, thus defining $S_m(K)$ for $m \leq \infty$.

By a *homomorphism (isomorphism)*[3]

$$F = (\mathfrak{h}, \mathfrak{g}, \mathfrak{f}) : S_m(K) \to S_m(K'), \tag{1.1}$$

where K' is a given complex, we mean a family of homomorphisms

$$\mathfrak{h}_{n+1} : H_{n+1} \to H_{n+1}', \quad \mathfrak{g}_n : \Gamma_n \to \Gamma_n', \quad \mathfrak{f}_n : \Pi_n \to \Pi_n'$$

such that $\mathfrak{b}\mathfrak{h} = \mathfrak{g}\mathfrak{b}$, $\mathfrak{i}\mathfrak{g} = \mathfrak{f}\mathfrak{i}$, $\mathfrak{j}\mathfrak{f} = \mathfrak{h}\mathfrak{j}$, where $\mathfrak{b} : H_{n+1}' \to \Gamma_n'$, etc., are the homomorphisms in $S(K')$. Let $\mathfrak{K}$ be any category[4] of (simply connected) complexes and homotopy classes of maps $K \to K'$, for every pair of complexes $K, K' \in \mathfrak{K}$. Let $\mathfrak{G}_m$ be the category in which the objects and mappings are the sequences, $S_m(K)$, and all homomorphisms, $S_m(K) \to S_m(K')$,

for every pair K, K' ϵ $\mathfrak{K}$. Then a homotopy class of maps, $K \to K'$, induces a unique homomorphism, $S_m(K) \to S_m(K')$, in such a way as to determine a functor $\mathfrak{K} \to \mathfrak{f}_m$. Thus $S_m(K)$ is a homotopy invariant and *a fortiori* a topological invariant of K. If a given homomorphism, $F:S_m(K) \to S_m(K')$, is the one induced by a map, $\phi:K \to K'$, we shall describe ϕ as a *geometrical realization* of F.

THEOREM 2. *Let dim K, dim $K' \leq m$. Then $\phi:K = K'$ if ϕ induces an isomorphism $F:S_m(K) \sim S_m(K')$.*

This follows at once from Theorem 3 in CH I.

Let $\lambda:S^3 \to S^2$ be a map which represents a fixed generator of $\pi_3(S^2)$ and let $\mu:S^2 \to K^2$ represent a given element $a \epsilon \Pi_2$. Then $\mu\lambda:S^3 \to K^2$ represents an element $\lambda(a) \epsilon \Gamma_3$. We shall describe equation (1.1) as a *proper* homomorphism (isomorphism) if, and only if,

$$\mathfrak{B}_3\lambda(a) = \lambda(\mathfrak{f}_2 a), \tag{1.2}$$

for every $a \epsilon \Pi_2$. Let equation (1.1) have a geometrical realization $\phi:K \to K'$. Then equation (1.1) is a proper homomorphism because $\phi(\mu\lambda) = (\phi\mu)\lambda$.

THEOREM 3. *Let dim $K \leq 4$. Then any proper homomorphism, $S_4(K) \to S_4(K')$, has a geometrical realization, $K \to K'$.*

We now anticipate the definition of $\Gamma(A)$ in §2 below and consider a purely algebraic (exact) sequence

$$S_4: \quad H_4 \overset{\mathfrak{b}}{\to} \Gamma_3 \overset{\mathfrak{t}}{\to} \Pi_3 \overset{\mathfrak{j}}{\to} \ldots \overset{\mathfrak{j}}{\to} H_3 \overset{\mathfrak{b}}{\to} o,$$

in which the (Abelian) groups are arbitrary except that

$$\theta:\Gamma(\Pi_2) \sim \Gamma_3, \qquad \Gamma_2 = 0.$$

The isomorphism θ is to be included as a component part of S_4. Let S_4', with groups H_{n+1}', Γ_n', Π_n', be a similar sequence. A *proper homomorphism (isomorphism)*, $S_4 \to S_4'$, shall mean the same as before, with equation (1.2) replaced by the condition

$$\mathfrak{B}_3\theta = \theta\mathfrak{B}:\Gamma(\Pi_2) \to \Gamma_3',$$

where θ means the same in S_4' as in S_4 and

$$\mathfrak{B}:\Gamma(\Pi_2) \to \Gamma(\Pi_2')$$

is the homomorphism induced by $\mathfrak{f}_2:\Pi_2 \to \Pi_2'$. By a *geometrical realization* of S_4 we shall mean a complex, K, such that $S_4(K)$ is properly isomorphic to S_4.

THEOREM 4. *The sequence S_4 has a geometrical realization, which is*

(a) *at most 4-dimensional if H_4 is free Abelian,*

(b) *a finite complex if each of H_2, H_3 and H_4 has a finite set of generators.*

Theorems 2, 3 and 4 show that $S_4(K)$ can be used to replace the more complicated "extended" cohomology ring[5] of K. Moreover they apply to infinite complexes and hence to universal covering complexes. Therefore it seems reasonable to hope that these theorems, in conjunction with the cohomology theory of abstract groups, may lead to similar theorems in case $\pi_1(K) \neq 1$.

2. *The Group* $\Gamma(A)$. Let A be an additive Abelian group and let wA be any aggregate which is in a (1-1) correspondence, $w:A \to wA$, with A. We define an (additive) group, $\Gamma(A)$, by means of the symbolic generators $w(a) \, \epsilon \, wA$ and the relations

$$w(a) = w(-a) \qquad (2.1a)$$

$$w(a + b + c) - w(b + c) - w(c + a) - w(a + b) + w(a) + \\ w(b) + w(c) = 0, \quad (2.1b)$$

together with the "trivial relations," $w(a) - w(a) = 0$. On writing $a = b = c = 0$ in (2.1b) we have $w(0) = 0$. Hence it follows from (2.1b), with $b = 0$, that $\Gamma(A)$ is Abelian. Let $b = (n - 1)a, c = -a$ $(n \geq 1)$. Then it follows from equation (2.1) and induction on n that $w(na) = n^2 w(a)$.

Let $\gamma(a)$ be the element of $\Gamma(A)$ which is represented by $w(a)$ and let

$$[a, b] = \gamma(a + b) - \gamma(a) - \gamma(b).$$

Then, given that addition is commutative, equation (2.1b) expresses the fact that $[a, b]$ is bilinear in a and b.

Let A be free Abelian and let $\{a_i\}$ be a set of free generators of A. Then $\Gamma(A)$ is freely generated by the elements $\gamma(a_i)$, $[a_j, a_k]$, for every a_i and every (unordered) pair of distinct elements $a_j, a_k \, \epsilon \, \{a_i\}$.

Let A, generated by a_1, be a finite, cyclic group of order m. Then $\Gamma(A)$ is generated by $\gamma(a_1)$ and is of order m or $2m$, according as m is odd or even.

Let A be the weak direct sum of a set of groups $\{A_i\}$. Let Γ be the weak direct sum of the groups $\Gamma(A_i)$ and the tensor products $A_j \rho A_k$, for every A_i and every (unordered) pair of distinct groups, A_j, A_k, in the set $\{A_i\}$. Then $\Gamma(A) \sim \Gamma$.

It follows that, if A is finitely generated, so is $\Gamma(A)$. Moreover the rank and invariant factors of $\Gamma(A)$ can be calculated from those of A and conversely. Also $a = 0$ if $[a, a'] = 0$ for every $a' \, \epsilon \, A$. Therefore the pairing $(A, A) \to \Gamma(A)$, in which $(a, a') = [a, a']$, is orthogonal.

It follows from the form of the relations (2.1) that a homomorphism $f:A \to A'$, into an additive Abelian group A', *induces* a homomorphism, $g:\Gamma(A) \to \Gamma(A')$, which is given by $g\gamma(a) = \gamma(fa)$. If A admits a group, Π_1, as a group of operators, so does $\Gamma(A)$, according to the rule $x\gamma = \gamma x(x \, \epsilon \, \Pi_1)$.

Let $A = \Pi_2(K)$ and let $\lambda(a)$ mean the same as in equation (1.2). Then $\lambda(a) = \lambda(-a)$ and[6]

$$\lambda(a + b) - \lambda(a) - \lambda(b) = [a, b],$$

where $[a, b] \in \Gamma_3(K)$ is the bilinear product, or commutator,[7] of a and b. Therefore the relations (2.1) are satisfied when w and $\equiv$ are replaced by λ and $\equiv$. Therefore a homomorphism, $\theta : \Gamma(\Pi_2) \to \Gamma_3$, is defined by $\theta\gamma(a) = \lambda(a)$.

Let $(\mathfrak{h}, \mathfrak{g}, \mathfrak{f}) : S_m(K) \to S_m(K')$ be a proper homomorphism and let $\mathfrak{g} : \Gamma(\Pi_2) \to \Gamma(\Pi_2')$ be the homomorphism induced by $\mathfrak{f}_2$. Then it follows from equation (1.2) and the relations $\mathfrak{g}\gamma = \gamma\mathfrak{f}_2$, $\theta\gamma = \lambda$, that

$$\theta\mathfrak{g} = \mathfrak{g}_3\theta : \Gamma(\Pi_2) \to \Gamma_3', \qquad (2.2)$$

where $\theta : \Gamma(\Pi_2') \to \Gamma_3'$ is also defined by $\theta\gamma = \lambda$. In particular let Π_1 be a group of homomorphisms of K onto itself (e.g., the covering group if K is a covering complex). Then each $x \in \Pi_1$ induces a proper automorphism $x : S_m(K) \approx S_m(K)$ and it follows from equation (2.2) that θ is an operator homomorphism.

THEOREM 5.[8] $\theta : \Gamma(\Pi_2) \approx \Gamma_3$.

3. · $\Gamma(A)$ and Cohomology. Let X be any topological space and let $H_n(G)$ be the Cech cohomology group of X, which is defined in terms of the nerves of all finite open coverings, with G as the (discrete) group of coefficients (we could equally well take closed coverings). We define the cup-product, $\mathbf{a} \cup \mathbf{b} \in H^{2n}\{\Gamma(A)\}$, of elements $\mathbf{a}, \mathbf{b} \in H^n(A)$, by means of the pairing $(a, b) \to [a, b]$, where $a, b \in A$.

THEOREM 6. *Let n be even. Then there is a natural homomorphism,*

$$h : \Gamma\{H^n(A)\} \to H^{2n}\{\Gamma(A)\},$$

such that $h[\mathbf{a}, \mathbf{b}] = \mathbf{a} \cup \mathbf{b}$ for any pair $\mathbf{a}, \mathbf{b} \in H^n(A)$.

We write $h\gamma = \mathfrak{p} : H^n(A) \to H^{2n}\{\Gamma(A)\}$ and call $\mathfrak{p}\mathbf{a}$ the *Pontrjagin square* of $\mathbf{a} \in H^n(A)$ (n is even). We have

$$\mathfrak{p}(\mathbf{a} + \mathbf{b}) = \mathfrak{p}\mathbf{a} + \mathfrak{p}\mathbf{b} + \mathbf{a} \cup \mathbf{b}, \qquad 2\,\mathfrak{p}\mathbf{a} = \mathbf{a} \cup \mathbf{a}. \qquad (3.1)$$

Thus $-\mathbf{a} \cup \mathbf{b}$ is a factor set, which measures the error made in supposing $\mathfrak{p}$ to be a homomorphism. Let $g : \Gamma(A) \to \Gamma(A')$ be the homomorphism induced by a homomorphism, $f : A \to A'$, into an additive Abelian group A'. Then f, g induce homomorphisms

$$f^n : H^n(A) \to H^n(A'), \qquad g^{2n} : H^{2n}\{\Gamma(A)\} \to H^{2n}\{\Gamma(A')\} \quad (3.2)$$

such that $\mathfrak{p}f^n = g^{2n}\mathfrak{p}$. If X is a finite polyhedron and if A is cyclic of even order, then $\mathfrak{p}$ is the same as in "SCP."

Let $X = K$ and let I_m be the group of integers, reduced mod. m. Let

$$H_n(m) = H_n(K, I_m), \qquad A_m = A - mA \qquad (m \geq 0).$$

Then the pairing $(A, I_m) \to A_m$, in which $(a, 1) \epsilon A_m$ is the residue class containing $a \epsilon A$, determines a homomorphism

$$u^*(m) : H^n(A) \to \text{Hom} \left\{ H^n(m), A_m \right\}. \tag{3.3}$$

If K has no $(n-1)$ dimensional torsion $u^*(0)$ is an isomorphism (onto).

Now take $A = H_n$ and let K be without $(n-1)$-dimensional torsion. Then

$$u^*(0) : H^n(H_n) \approx \text{Hom} (H_n, H_n)$$

and we identify each element $\mathfrak{K} \epsilon H^n(H_n)$ with $u^n(0)\mathfrak{K}$. Thus $H^n(H_n)$ becomes the additive group of the ring, E_n, of endomorphisms of H_n. Let $f^n(e), g^{2n}(e)$ denote f^n, g^{2n} in equation (3.2) when $A = A' = H_n$ and $f = e \epsilon E_n$. Then it follows from the way in which e induces $f^n(e)$ that $f^n(e)e' = ee'$ $(e' \epsilon E_n)$. Since $\mathfrak{P}f^n = g^{2n}\mathfrak{P}$ we have

$$\mathfrak{P}(ee') = g^{2n}(e)\mathfrak{P}e', \qquad \mathfrak{P}e = g^{2n}(e)\mathfrak{P}(1), \tag{3.4}$$

where $1 \epsilon E_n$ is the identity. Thus $\mathfrak{P}$ is determined by the map $e \to g^{2n}(e)$ and by $\mathfrak{P}(1)$.

Now let K be a finite (simply connected) complex of arbitrary dimensionality. We make the natural identification $\Pi_2 = H_2$ and, using Theorem . 5, we identify each $\gamma \epsilon \Gamma(H_2)$ with $\theta\gamma \epsilon \Gamma_2$. Also K has no 1-dimensional torsion and we identify each $\mathfrak{K} \epsilon H^2(H_2)$ with $u^2(0)\mathfrak{K} \epsilon E_2$. Then equation (3.3), with $n = 4$, $A = \Gamma_3$, becomes

$$u(m) = u^4(m) : H^4(\Gamma_3) \to \text{Hom} \left\{ H_4(m), \Gamma_{3, m} \right\}.$$

The homomorphism $\gamma : C_4(K) \to \Gamma_3$, which is defined on page 85 of "SCP," induces what we call the secondary *modular* boundary homomorphism,[9]

$$\mathfrak{b}(m) \epsilon \text{Hom} \left\{ H_4(m), \Gamma_{3, m} \right\},$$

and $\mathfrak{b}(0)$ is the same as $\mathfrak{b}_4$ in $S_4(K)$.

THEOREM 7. $\mathfrak{b}(m) = u(m)\mathfrak{P}(1) \qquad (m \geq 0)$.

4. *The Calculation of $S_4(K)$.* The group Π_3, in $S_4(K)$, is an extension of H_3 by $G = \Gamma_3 - \mathfrak{b}H_4$ and $S_4(K)$ is determined, up to a proper isomorphism, by H_2, H_3, H_4, the homomorphism $\mathfrak{b}_4$ and the element of $H^2(H_3, G)$ which determines the equivalence class of the extension Π_3. Let K be a finite, simplicial complex. Then it will be shown how these items may be calculated (constructively) with the help of Theorems 5 and 7. This construction does not provide a finite algorithm for deciding whether or not $S_4(K)$ is properly isomorphic to $S_4(K')$. Some of the difficulties inherent in this question are indicated on page 88 of "SCP."

5. A_n^2-*polyhedra.* Let $\pi_r(K) = 0$ for $r = 1, \ldots, n - 1$, where $n > 2$. In this case we may identify Γ_{n+1} with[10] $H_n(2)$ and $\mathfrak{h}_{n+2}$ determines a homomorphism, $\mathfrak{h}(2):H_{n+2}(2) \to H_n(2)$, which is the dual of[11] $Sq_{n-2}:H^n(2) \to H^{n+2}(2)$. The structure of Π_{n+2}, as an extension of H_{n+1} by $H_n(2)$, is determined by $\mathfrak{h}(2)$. Thus $S_{n+2}(K)$ is determined, up to a proper isomorphism, by the co-homology system $H(K)$, or by the analogous system of homology groups,[12] in which $\mathfrak{h}(2)$ plays the part of Sq_{n-2}.

[1] All our complexes will be simply connected CW-complexes, as defined in §5 of J. H. C. Whitehead, "Combinatorial Homotopy I," *Bull. Am. Math. Soc.*, 55, 213–245 (1949). This paper will be referred to as CH I.

[2] In the light of this theorem a J_m-complex, K, as defined in CH I, is seen to be one such that $\mathfrak{f}_n:\pi_n(\bar{K}) \approx H_n(\bar{K})$, if $n \leq m$, and $\mathfrak{f}_{m+1}$ is onto, where $\bar{K}$ is the universal covering complex of K. This, and the other theorems stated here, will be proved in a paper which is to appear in the *Annals of Mathematics*.

[3] An isomorphism will always mean an isomorphism onto.

[4] Cf. Eilenberg, S., and MacLane, Saunders, "General Theory of Natural Equivalences," *Trans. Am. Math. Soc.*, 58, 231–294 (1945).

[5] See Whitehead, J. H. C., "On Simply Connected, 4-Dimensional Polyhedra," *Comm. Math. Helvetici*, 22, 48–92 (1949). This paper will be referred to as "SCP."

[6] See equation (7.3) in Whitney, Hassler, "Relations Between the Second and Third Homotopy Groups of a Simply Connected Space," *Ann. Math.*, 50, 180–202 (1949).

[7] Cf. Fox, R. H., "Homotopy Groups and Torus Homotopy Groups," *Ibid.*, 49, 471–510 (1948).

[8] Cf. Hirsch, G., "Sur le troisieme groupe d'homotopie des polydères simplement connexe," *C. R. Acad. Sci. Paris*, 228, 1920–1922 (1949), in case K is finite and without 2-dimensional torsion. Hirsch's representation of $\Gamma_3 - \mathfrak{h}H_4$ can be obtained from Theorem 5 and Theorem 7 below.

[9] In the forthcoming paper $\mathfrak{h}(m)$ is defined more generally and is shown to be natural.

[10] See Whitehead, J. H. C., "The Homotopy Type of a Special Kind of Polyhedron," *Ann. Soc. Polonaise Math.*, 21, 176–186 (1949); also Whitehead, G. W., "On spaces with vanishing low-dimensional homotopy groups", *Proc. Nat. Acad. Sci.*, 34, 207–211 (1948).

[11] Steenrod, N. E., "Products of Cocycles and Extensions of Mappings," *Ann. Math.*, 48, 290–320 (1947).

[12] See a forthcoming paper by P. J. Hilton.

THEOREMS ON QUADRATIC PARTITIONS

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If p is a prime of the form $3f + 1$, the diophantine equation $4p = a^2 + 3b^2$ has a unique solution in a and b with $a \equiv 1 \pmod 3$ and $b \equiv 0 \pmod 3$. About forty years ago von Schrutka[1] derived the formula $a = 1 + \phi_2(4)$, where $\phi_2(n)$ is the Jacobstahl sum defined by

$$\phi_k(n) = \sum_{h=0}^{p-1} \left(\frac{h}{p}\right)\left(\frac{h^k + n}{p}\right), \tag{1}$$

the symbol (h/p) denoting the quadratic character of h with respect to p. Recently, the following analogous result has been found by E. Lehmer.[1] If p is a prime of the form $5f + 1$, then the value of x in the pair of diophantine equations $16p = x^2 + 50u^2 + 50v^2 + 125w^2$ and $xw = v^2 - 4uv - u^2$ is given by $x = 1 + \phi_5(4)$ with $x \equiv 1 \pmod 5$.

It is interesting to ask if a similar formula holds for a prime p of the form $7f + 1$. The purpose of this note is to point out that this is not the case; we offer in its place the result stated in Theorem 2. Our method is based on the theory of cyclotomy.

Let g be a primitive root of a prime p of the form $ef + 1$. A number N, prime to p, is congruent to a power of g:

$$N \equiv g^{es + h} \pmod{p}, \quad 0 \leq s \leq f - 1, \quad 0 \leq h \leq e - 1.$$

For fixed h and k, $0 \leq h, k \leq e - 1$, the cyclotomic number (h, k) is defined as the number of sets of values of s and t, each chosen from $0, 1, \ldots, f - 1$ for which the congruence

$$g^{es + h} \equiv 1 + g^{et + k} \pmod{p}$$

holds. The numbers (h, k) satisfy, among others, the following properties:[2]

$$(h, k) = (k, h) = (e - k, h - k), \quad e \text{ odd}, \tag{2}$$

and

$$\sum_{k=0}^{e-1} (k, h) = f - n_k, \quad k = 0, 1, \ldots, e - 1, \tag{3}$$

where $n_k = 1$ if $k = 0$ and $n_k = 0$ if $k \neq 0$.

Let β denote a primitive eth root of unity. The numbers prime to p may be distributed into e classes according to the residue classes $(\mod e)$ of their indices to the base g. We accordingly define the following extension of the Legendre symbol:

$$\left(\frac{N}{p}\right)_e = \beta^{\text{ind } N}, \tag{4}$$

for an integer N prime to p. For an integer N divisible by p, we put $(N/p)_e = 0$. If N is an eth residue of p, that is, if the congruence $x^e \equiv N \pmod p$, $p \nmid N$, is solvable, then $(N/p)_e = 1$, regardless of the value of g. Otherwise, the value of $(N/p)_e$ depends on the primitive root taken as base.

For any pair of integers m, n define the cyclotomic function $R(m, n)$ by means of the equation

$$R(m, n) = \sum_{t=1}^{p-2} \beta^{m \text{ ind } t - (m + n) \text{ ind } (1 + t)}. \tag{5}$$

From the theory of cyclotomy[4] we have the following fundamental property . of $R(m, n)$. If no one of the integers m, n and $m + n$ is divisible by e, then

$$R(m, n)R(-m, -n) = p. \tag{6}$$

·Furthermore, the value of $R(-m, -n)$ may be derived from the value of $R(m, n)$ by replacing β by β^{-1}.

In this note[5] we shall investigate the cases $e = 3$ and $e = 7$. Assume first that $e = 3$. In this case, the formulas in equation (2) may be expressed schematically by means of the matrix

$$\begin{vmatrix} A & B & C \\ B & C & D \\ C & D & B \end{vmatrix}, \tag{7}$$

in which the element in the hth row and kth column, h, $k = 0, 1, 2$, represents the value of the cyclotomic number (h, k). In terms of this notation the equations in equation (3) become

$$A + B + C = f - 1, \quad B + C + D = f. \tag{8}$$

Let $f(k)$ denote the number of distinct solutions of the congruence

$$x^2 + x \equiv k \pmod{p}. \tag{9}$$

Then the sum $\sum_\alpha f(\alpha)$, where α runs over the $(p - 1)/3$ cubic residues of p, is equal to the number of values of x, $1 \leq x \leq p - 1$, for which $x^2 + x$ is a cubic residue of p, that is, for which $(x/p)_3((x + 1)/p)_3 = 1$. In view of equation (7), this number is equal to $(0, 0) + (1, 2) + (2, 1) = A + 2D$. It follows that

$$\sum_{s=0}^{p-1} f(s^2) = f(0) + 3 \sum_\alpha f(\alpha) = 2 + 3(A + 2D). \tag{10}$$

In equation (5) take $m = n = 1$, and $\beta = \omega$, a primitive cube root of unity. Using equation (4), we get

$$R(1, 1) = \sum_{t=1}^{p-2} \omega^{\text{ind } t - 2 \text{ ind } (1+t)} = \sum_{t=1}^{p-1} \left(\frac{t}{p}\right)_3 \left(\frac{1+t}{p}\right)_3.$$

Putting $R(1, 1) = r + s\omega + t\omega^2$, we deduce from equations (6) and (7) that $4p = a^2 + 3b^2$, where

$$a = 2r - s - t, \qquad b = s - t, \qquad (11)$$

and

$$r = A + 2D, \qquad s = 3B, \qquad t = 3C. \qquad (12)$$

Combining equations (8), (10), (11) and (12), we obtain

$$a = -p + 2 + 3r = -p + \sum_{s=0}^{p-1} f(s^3). \qquad (13)$$

Let r_v be a number not divisible by p such that $(r_v/p)_3 = \omega^v, v = 0, 1, 2.$
Then we may show in a similar fashion that

$$b = \frac{1}{3} \left(\sum_{s=0}^{p-1} f(r_1 s^3) - \sum_{s=0}^{p-1} f(r_2 s^3) \right). \qquad (14)$$

Note that equation (13) implies that the sign of a is such that the congruence $a \equiv 1 \pmod 3$ is satisfied, whereas the sign of b in equation (14) depends on the primitive root g employed.

We have thus proved the following theorem.

THEOREM 1.   *The values of a and b in the equation $4p = a^2 + 3b^2$, where $p = 3f + 1$ is a prime, are given by equations (13) and (14), where the sign of a is determined by the condition $a \equiv 1 \pmod 3$, and $f(k)$ denotes the number of distinct solutions of the congruence (9).*

As an application we observe that the formula $f(k) = 1 + ((4k + 1)/p)$ lead at once to the theorem of von Schrutka stated in the introduction.

We turn now to the case $e = 7$.   In this case the formulas in equation (2) yield the matrix

$$\begin{vmatrix} A & B & C & D & E & F & G \\ B & G & H & I & J & K & H \\ C & H & F & K & L & L & I \\ D & I & K & E & J & L & J \\ E & J & L & J & D & I & K \\ F & K & L & L & I & C & H \\ G & H & I & J & K & H & B \end{vmatrix}, \qquad (15)$$

in which the letter in the hth row and kth column, $h, k = 0, 1, 2 \ldots, 6$, represents the value of (h, k).   In terms of this notation the equations in equation (3) reduce to

$$\begin{aligned} A + B + C + D + E + F + G &= f - 1, \\ B + G + 2H + I + J + K &= f, \\ C + F + H + I + K + 2L &= f, \\ D + E + I + K + 2J + L &= f. \end{aligned} \qquad (16)$$

Let $g(k)$ denote the number of distinct solutions of the congruence

$$x^3 + x^2 \equiv k \pmod{p}. \tag{17}$$

The sum $\sum_{\alpha} g(\alpha)$, where α runs over the $(p-1)/7$ incongruent seventh-power residues with respect to the modulus p, is equal to the number of values of x, $1 \le x \le p - 1$, for which $x^3 + x^2$ is a seventh-power residue of p, that is, for which $(x/p)_7^3 ((x+1)/p)_7 = 1$. In view of equation (15), this number is equal to $(0, 0) + (1, 5) + (2, 3) + (3, 1) + (4, 6) + (5, 4) + (6, 2) = A + 3I + 3K$. It follows that

$$\sum_{s=0}^{p-1} g(s^7) = g(0) + 7 \sum_{\alpha} g(\alpha) = 2 + 7(A + 3I + 3K). \tag{18}$$

In equation (5) take $m = 1$, $n = 2$, and $\beta = \theta$, a primitive seventh root of unity. Making use of equation (4), we obtain

$$R(1, 2) = \sum_{i=1}^{p-2} \theta^{\text{ind } i - 3 \text{ ind } (1+i)} = \sum_{i=1}^{p-1} \left(\frac{i}{p}\right)_7 \left(\frac{1+i}{p}\right)_7^4. \tag{19}$$

It follows from equations (15) and (19) that $R(1, 2)$ may be written in the form $R(1, 2) = r + s(\theta + \theta^2 + \theta^4) + t(\theta^3 + \theta^5 + \theta^6)$, where

$$\begin{aligned} r &= A + 3I + 3K, \\ s &= B + C + E + H + J + K + L, \\ t &= D + F + G + H + I + J + L. \end{aligned} \tag{20}$$

Applying equation (6), we get also $4p = a^2 + 7b^2$, where

$$a = 2r - s - t, \qquad b = s - t. \tag{21}$$

Combining equations (16), (18), (20) and (21) we obtain

$$3a = -p + 2 + 7r = -p + \sum_{s=0}^{p-1} g(s^7). \tag{22}$$

Let r_v be a number not divisible by p such that $(r_v/p)_7 = \theta^v$, $v = 0, 1$ $2, \ldots, 6$. Then we may show in a similar fashion that

$$b = \frac{1}{7} \left(\sum_{s=0}^{p-1} g(r_1 s^7) - \sum_{s=0}^{p-1} g(r_6 s^7) \right). \tag{23}$$

The formula in equation (22) implies that the sign of a is such that the congruence $a \equiv 5 \pmod 7$ is satisfied; the sign of b, however, depends on the choice of the primitive root g. We have therefore proved the following theorem.

THEOREM 2. *The values of a and b in the equation $4p = a^2 + 7b^2$, where $p = 7f + 1$ is a prime, are given by equations (22) and (23) ,where the sign*

of a is determined by the condition $a \equiv 5 \pmod{7}$, and $g(k)$ denotes the number of distinct solutions of the congruence (17).

The following result due to Dickson[6] may be used as a criterion for determining the value of $g(k)$.   Let p be a prime >3, and let $R = -4a^3 - 27b^2$ be the discriminant of the cubic congruence

$$x^3 + ax + b \equiv 0 \pmod{p}.$$

This congruence has a single integral root if and only if R is a quadratic non-residue of p; it has three distinct integral roots if and only if R is the residue of a square $81c^2 \neq 0$ and $e = \frac{1}{2}(-b + c\sqrt{-3})$ is the residue of the cube of a number $u + v\sqrt{-3}$ in which u and v are integers; it has no integral root if and only if R is a quadratic residue and e is not the residue of such a cube.   In order to apply this theorem it is only necessary to observe that for $k \neq 0$, $g(k)$ is the number of solutions of the congruence $x^3 - \bar{k}x - \bar{k} \equiv 0 \pmod{p}$, where $k\bar{k} \equiv 1 \pmod{p}$.

Unfortunately, Dickson's criterion does not lead to a simple formula expressing the value of a in Theorem 2 in terms of the Jacobstahl sum defined in equation (1).

[1] Von Schrutka L., *J. Reine Angew. Math.*, 140, 252–265 (1911).   Von Schrutka's result has been rediscovered by S. Chowla, *Proc. Lahore Philos. Soc.*, 7, 2 pp. (1945).

[2] Lehmer, E., *Bull. Am. Math. Soc.*, 55, 62–63 (1949), Abstract No. 72.

[3] Bachmann, P., *Die Lehre von der Kreisteilung*, 2nd ed., B. G. Teubner, 1921, pp. 201–203.

[4] *Ibid.*, p. 123.   Bachmann's formula (3) is equivalent to our formula (6).

[5] For a treatment of the case $e = 5$, see a forthcoming paper by the author in *Duke Math. J.*

[6] Dickson, L. E., *Bull. Am. Math. Soc.*, 13, 1–8 (1906).

A NOTE ON TIIE MEAN SQUARE VELOCITY IN STELLAR STATISTICS

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1. *Introduction.*—In a recent paper Chandrasekhar and Münch[1] have obtained a formula connecting the mean rotational velocity (or its higher moments) of stars with the mean value (or the higher moments) of a directly observable quantity $v \sin i$, where i is the inclination of axis of rotation of the star with the line of sight. The merit of this formula lies in the fact that we can compute a true mean value without any knowledge of the true distribution of the rotational velocity. Here we shall apply this same idea to a different problem in stellar statistics.

Considering stars in a certain region of the sky, we can observe the radial velocity and the two components of tangential velocity;[2] we can therefore obtain from the observational data the mean square velocity components in three mutually perpendicular directions. The question we shall consider is the method by which these means can be converted into mean square velocity components in three principal directions of stellar motion, for example, along the three principal axes of the velocity ellipsoid.[3] Before going into the problem just mentioned, we shall first formulate the basic idea underlying Chandrasekhar and Münch's paper in a more general form.

2. *A Statistical Relation.*—Let $\phi(y)$ be the observed distribution with respect to a directly observable quantity y, which is a function of two independent physical quantities x and t; thus

$$y = g(x, t). \tag{1}$$

Let the probability that t lies between t and $t + dt$ be $w(t)$. Then the probability distribution, $f(x)$, of the physical quantity x is evidently related to $\phi(y)$ by an integral equation which can be derived in the following manner:

Let y_0 and y_1 denote the least and the greatest possible values of y, respectively. Then

$$g(x, t) = \text{constant} = a \ (y_0 \leqq a \leqq y_1) \tag{2}$$

represents a one-parameter family of curves in the (x, t) plane. If this family of curves covers continuously a certain domain S in the (x, t) plane without overlapping, then the integral equation relating $\phi(y)$ and $f(x)$ can be verified to be

$$\phi(y) = \int_\alpha^\beta f(x)w(t) \left| \frac{\partial g}{\partial t} \right|^{-1} dx \tag{3}$$

β and α being the boundaries of the domain in the (x, y) plane resulting from the transformation of the (x, t) plane according to (1); hence both β and α may be functions of y. The mapping of the (x, t) plane on the (x, y) plane by (1) is assumed to be one-one and continuous. From (3) we obtain the following relation:

$$\overline{y^n} = \int_{y_0}^{y_1} y^n \phi(y) \, dy = \int_S \int g^n(x, t)f(x)w(t) \, dx \, dt. \tag{4}$$

If the observed quantity y is a function of more than two variables,

$$y = g(x, t_1, \ldots t_n)$$

and if the probability distribution for $x, t_1, \ldots t_n$ are $f(x), w_1(t_1), \ldots w_n(t_n)$ respectively, then

$$\overline{y^n} = \int \ldots \int g^n(x, t_1, \ldots t_n)f(x)w_1(t_1)\ldots w_n(t_n) \, dx \, dt_1 \ldots dt_n. \tag{5}$$

It is worthy of notice that $f(x)$ may also involve the variables t_i.

If the variables in $g(x, t)$ are separable, i.e.,

$$y = g(x, t) = g_1(x)g_2(t)$$

then

$$y^n = \int g_1{}^n(x)f(x) \, dx \int g_2{}^n(t)w(t) \, dt \tag{6}$$

and we obtain the relation

$$\overline{y^n} = \overline{x^n} \int g_2{}^n(t)w(t) \, dt \tag{7}$$

for $g_1(x) = x$. An example of (7) is the case considered by Chandrasekhar and Münch; thus, for their problem

$$g_2(t) = w(t) = \sin t.$$

Therefore

$$\overline{y^n} = \overline{x^n} \int_0^{\pi/2} \sin^{n+1} t \, dt.$$

3. Relations between Mean Square Velocities.—Consider a celestial sphere with the observer at the center O. Let OV represent the direction of space velocity of a star at S. Choosing a coordinate system with OX pointing in the direction of star streaming and OZ in the direction of minimum mean speed, we measure the spherical coordinates θ_i and φ_i from the OX-axis and the OXZ-plane, respectively. We let the coordinates of S be (θ_1, φ_1) and of V, (θ_2, φ_2). Furthermore we denote the arc SV by Θ and the angle between the planes OSV and OSX by ϕ.

If $\vec{v}$ denotes the space velocity, the components v_1, v_2 and v_3 of $\vec{v}$ resolved along the radial direction OS, and in the two mutually perpendicular directions in the tangential plane are, respectively,

$$v_1 = v \cos \Theta, \qquad v_2 = v \sin \Theta \cos \phi, \qquad v_3 = v \sin \Theta \sin \phi. \quad (8)$$

According to the general formula (5), the observed mean square velocities (i.e., $\overline{v_1^2}$, $\overline{v_2^2}$ and $\overline{v_3^2}$) are related to the distribution $f(v)$ by the following equations

$$\overline{v_1^2} = \frac{1}{4\pi} \int\int\int v^2 \cos^2 \Theta \sin \Theta \, f(v) \, dv \, d\Theta \, d\phi, \quad (9)$$

$$\overline{v_2^2} = \frac{1}{4\pi} \int\int\int v^2 \sin^3 \Theta \cos^2 \phi \, f(v) \, dv \, d\Theta \, d\phi, \quad (10)$$

$$\overline{v_3^2} = \frac{1}{4\pi} \int\int\int v^2 \sin^3 \Theta \sin^2 \phi \, f(v) \, dv \, d\Theta \, d\phi. \quad (11)$$

If the velocity distribution function $f(v)$ were independent of the direction (spherical distribution) equations (9–11) lead to the well-known result

$$\overline{v_1^2} = \overline{v_2^2} = \overline{v_3^2} = \tfrac{1}{3} \overline{v^2}.$$

In fact, as is well known, the velocity distribution does depend upon the direction. Let u_1, u_2 and u_3 be the velocity components along the three coordinate axes, and l, m and n be the direction cosines of OV then

$$v = lu_1 + mu_2 + nu_3$$

and

$$\overline{v^2} = l^2\overline{u_1^2} + m^2\overline{u_2^2} + n^2\overline{u_3^2} \quad (12)$$

if we assume that there is no correlation between two different components so that $\overline{u_i u_j} = 0$ for $i \neq j$. This is the only assumption we need to make and it is of such a general nature that it will include all cases of practical interest. Since

$$l = \cos \theta_2, \qquad m = \sin \theta_2 \sin \varphi_2, \qquad n = \sin \theta_2 \cos \varphi_2, \quad (13)$$

equations (9–11) can be rewritten in the forms

$$\overline{v_1^2} = A_1\overline{u_1^2} + B_1\overline{u_2^2} + C_1\overline{u_3^2}, \tag{14}$$

$$\overline{v_2^2} = A_2\overline{u_1^2} + B_2\overline{u_2^2} + C_2\overline{u_3^2}, \tag{15}$$

$$\overline{v_3^2} = A_3\overline{u_1^2} + B_3\overline{u_2^2} + C_3\overline{u_3^2}, \tag{16}$$

where A_1, B_1, ..., etc., denote the expressions which can be obtained by inserting (12) in (9–11). Thus,

$$4\pi A_1 = \int\int \cos^2\Theta \sin\Theta \cos^2\theta_2 \, d\Theta \, d\phi, \text{ etc.,}$$

where the integration is effected over the entire spherical surface. The various integrations can be carried out if use is made of the relations

$$\sin\theta_2 \cos\varphi_2 = (\cos\Theta \sin\theta_1 - \sin\Theta \cos\theta_1 \cos\phi) \cos\varphi_1$$
$$- \sin\Theta \sin\phi \sin\varphi_1 \quad (17)$$

and

$$\sin\theta_2 \sin\varphi_2 = (\cos\Theta \sin\theta_1 - \sin\Theta \cos\theta_1 \cos\phi) \sin\varphi_1$$
$$+ \sin\Theta \sin\phi \cos\varphi_1 \quad (18)$$

which readily follow from the standard formulas of spherical trigonometry.

In this manner we obtain

$$
\left.
\begin{aligned}
&15A_1 = 1 + 2\cos^2\theta_1, \qquad 15A_2 = 1 + 2\sin^2\theta_1, \quad 15A_3 = 1 \\
&15B_1 = 1 + 2\sin^2\theta_1 \sin^2\varphi_1, \qquad 15B_2 = 1 + 2\cos^2\theta_1\sin^2\varphi_1, \\
&\qquad\qquad\qquad\qquad\qquad 15B_3 = 1 + 2\cos^2\varphi_1 \\
&15C_1 = 1 + 2\sin^2\theta_1\cos^2\varphi_1, \qquad 15C_2 = 1 + 2\cos^2\theta_1\cos^2\varphi_1, \\
&\qquad\qquad\qquad\qquad\qquad 15C_3 = 1 + 2\sin^2\varphi_1.
\end{aligned}
\right\} \quad (19)
$$

Thus equations (14–16) together with (19) determine $\overline{u_i^2}$ completely from the observed quantities $\overline{v_i^2}$, if we observe only in the immediate neighborhood of a definite point in the sky.

It is also of interest to notice that

$$\overline{v_1^2} + \overline{v_2^2} + \overline{v_3^2} = \tfrac{1}{3}(\overline{u_1^2} + \overline{u_2^2} + \overline{u_3^2}), \tag{20}$$

a property independent of (θ_1, φ_1).

If furthermore we observe stars uniformly all over the sky (as for example a definite number of stars per unit area), the mean squares of the observed data will be averages with respect to θ_1 and φ_1 as well as with respect to Θ and ϕ. Multiplying equations (14–16) by $\sin\theta_1 \, d\theta_1 \, d\varphi_1/(4\pi)$ and integrating over the entire celestial sphere, we finally get

$$45\overline{v_1^2} = 5\overline{u_1^2} + 5\overline{u_2^2} + 5\overline{u_3^2}, \tag{21}$$

$$45\overline{v_2^2} = 7\overline{u_1^2} + 4\overline{u_2^2} + 4\overline{u_3^2}, \tag{22}$$

$$45\overline{v_3^2} = 3\overline{u_1^2} + 6\overline{u_2^2} + 6\overline{u_3^2}. \tag{23}$$

As $\overline{u_1^2} \geq \overline{u_2^2} \geq \overline{u_3^2}$, it follows from these equations that $\overline{v_3^2} \geq \overline{v_1^2} \geq \overline{v_2^2}$; this inequality must be clearly valid since v_1 is always in the direction of maximum mean motion.

Similar results can be obtained for the components of the tangential velocity v_2' and v_3' in the direction of minimum mean motion and in a direction perpendicular to it. For this purpose we choose the directions of minimum mean motion as the X-axis from which θ_1 and θ_3 are measured. Evidently v_1' does not change its meaning but still represents the radial velocity. The final results can be written down by a cyclic change of the indices. Thus for a definite region of the sky we have

$$\overline{v_i'^2} = A_i\overline{u_2^2} + B_i\overline{u_1^2} + C_i\overline{u_3^2} \quad (i = 1, 2, 3), \tag{24}$$

and the equations similar to (21–23) will be

$$45\overline{v_1'^2} = 5\overline{u_1^2} + 5\overline{u_1^2} + 5\overline{u_2^2}, \tag{25}$$

$$45\overline{v_2'^2} = 7\overline{u_2^2} + 4\overline{u_1^2} + 4\overline{u_2^2}, \tag{26}$$

$$45\overline{v_3'^2} = 3\overline{u_2^2} + 6\overline{u_1^2} + 6\overline{u_2^2}. \tag{27}$$

Hence in this case we have

$$\overline{v_3'^2} \geq \overline{v_1'^2} \geq \overline{v_2'^2}.$$

We can, of course, also express our results by choosing the direction of u_3 as the X-axis; the final equations will be obtained by a further cyclic change of indices in u_i.

It is of course clear that analogous relations between $\overline{v_i^{2n}}$ and $\overline{u_i^{2n}}$ can also be derived.

4. On a Possible Application of the Formulas of the Previous Section.— If the direction of star streaming and the direction of minimum mean motion are assumed to be known, we may compute the axes of the velocity ellipsoid by means of the relations derived. Among the three components of velocity v_1, v_2 and v_3 of the stars, the radial velocity v_1 is the one which is most accurately determined. Therefore in practical applications it will be advisable to utilize only the observational material on the radial velocities. This can be achieved by considering (14) only without using (15, 16).

Dividing the whole sky into small regions of, say 100 square degrees we use for each region the equation

$$15\overline{v_{\text{rad}}^2} = (1 + 2\,\overline{\cos^2\theta_1})\overline{u_1^2} + (1 + 2\,\overline{\sin^2\theta, \sin^2\varphi_1})\overline{u_2^2} + (1 + 2\,\overline{\sin^2\theta_1\cos^2\varphi_1})\overline{u_3^2}. \tag{28}$$

Equation (28) is simply (14) averaged over the small region. We shall thus obtain as many equations as the number of regions into which the whole sky has been divided. (In practice we may choose only those regions where the number of stars with measured radial velocities exceeds a certain lower

limit and discard others for statistical reasons.)   A least squares solution of these equations will determine the three unknowns $\overline{u_1{}^2}$, $\overline{u_2{}^2}$ and $\overline{u_3{}^2}$.   In forming the normal equations the number of stars in each region should enter as the weight factor.

This short note was inspired by Professor S. Chandrasekhar's stimulating colloquium on the paper cited previously; thus I should like to express my sincere thanks to him.   It is also my pleasure to put on record that equation (7) was independently noticed by both Dr. A. Brown and D. E. Osterbrock.   To both of them I am also indebted for some valuable discussions.

[1] Chandrasekhar, S., and Münch, G., to appear in January, 1950, issue of *Astrophys. J.*

[2] Due to the statistical nature of the present problem, it suffices to use the spectroscopic parallax in order to obtain the tangential velocity.

[3] For the terminology used in the present communication see Chandrasekhar, *Principles of Stellar Dynamics*, University of Chicago Press, 1942.

RADIAL OSCILLATIONS OF COMPRESSIBLE GAS SPHERES*

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The problem of radial oscillations of compressible gas spheres in hydrostatic equilibrium has so far been solved analytically for four different model configurations, three of which were discovered by Sterne,[1] while the fourth one was recently added by Prasad.[2]   In each case, the respective configuration proved to be capable of oscillating in a discrete set of frequencies dependent on the mean density and the ratio of specific heats of the material constituting the configuration.   The writer has, however, recently pointed out[3] that the assumptions made by Sterne and Prasad concerning the structure of their models were, in each case, such as to reduce the differential equations to the hypergeometric form; the discrete character of the frequency spectra followed as a consequence of the fact that the respective hypergeometric series of unit radius were found to be divergent and had to be reduced to polynomials in order to maintain no variation in pressure over the free surface.   Although the four models considered by Sterne and Prasad are characterized by outwardly very different distribution of density in their interiors, mathematically they all belong to the same type.   This similarity, in turn, prompts us to inquire as to the possible existence of other models, of different constitution, whose eigen-amplitudes of radial oscillations may also be expressible in terms of hypergeometric series.   Are the four known models the only ones possessing this property, or are there others of this class which have so far escaped discovery?

The object of this investigation will be to supply an exhaustive answer to this question and to provide a complete enumeration of the respective configurations. It will be shown that, for finite values of central condensation (i.e., of the ratio of the mass stored at the center to that of the whole configuration), no models other than those investigated by Sterne and Prasad exist for which the amplitudes of small radial oscillations are of the hypergeometric type. If, however, the degree of central condensation is allowed to increase without limit, a whole new family of such models is found to exist which is characterized by a partly discrete and partly continuous spectrum of the frequencies of free oscillation. This latter branch of the family represents the first known instance of compressible gas spheres, in hydrostatic equilibrium, which can perform small radial oscillations in *any* frequency.

1. *Equations of the Problem.*—As is well known[4] the differential equation governing the variation of the amplitude $\xi(x)$ of small adiabatic radial oscillations in the interior of a gas configuration in hydrostatic equilibrium can be reduced to the form

$$\frac{d^2\xi}{dx^2} + \left\{\frac{4}{x} + \frac{d \log P}{dx}\right\} \frac{d\xi}{dx} + \left\{\frac{a}{x} - \frac{n^2 R}{\gamma g}\right\} \frac{d \log P}{dx} \, \xi = 0, \qquad (1)$$

where P denotes the pressure; g, the gravity; n, the frequency of the oscillation; γ, the ratio of specific heats; $a = 3 - (4/\gamma)$; and $x = r/R$, r being the distance of any arbitrary point from the center of a spherically symmetrical configuration of radius R. Let us assume now that, by hypothesis,

$$\xi(x) \propto x^c F(\alpha, \beta, \gamma, ax^b), \qquad (2)$$

where F denotes the ordinary hypergeometric series and a, b, c, α, β, γ are arbitrary constants. The function on the right-hand side of (2)—let us call it y—is known to satisfy the equation

$$\frac{d^2y}{dx^2} + \frac{apx^b + q}{x(ax^b - 1)} \frac{dy}{dx} + \frac{arx^b + s}{x^2(ax^b - 1)} \, y = 0, \qquad (3)$$

where

$$
\begin{aligned}
\alpha &= \frac{1}{b} \left\{ \frac{p+q}{2} + \sqrt{\left(\frac{q+1}{2}\right)^2 + s} + \sqrt{\left(\frac{p-1}{2}\right)^2 - r} \right\} \\
\beta &= \frac{1}{b} \left\{ \frac{p+q}{2} + \sqrt{\left(\frac{q+1}{2}\right)^2 + s} - \sqrt{\left(\frac{p-1}{2}\right)^2 - r} \right\} \\
\gamma &= 1 + \frac{2}{b} \sqrt{\left(\frac{q+1}{2}\right)^2 + s},
\end{aligned}
\right\} \qquad (4)
$$

while c is the root of the quadratic equation

$$c^2 - (q + 1)c - s = 0. \tag{5}$$

If there is to be no displacement at the center—which is one of the boundary conditions of our problem—only positive roots of this equation are of physical interest.

The series on the right-hand side of (2) is known to converge absolutely and uniformly for $ax^b < 1$ or, should $ax^b = 1$, if $\alpha + \beta < \gamma$. It will be divergent if $ax^b > 1$ or, for $ax^b = 1$, if $\alpha + \beta \geqslant \gamma$. An appeal to equations (4) discloses that, if $ax^b = 1$, our series will be divergent if

$$p + q \geqslant b, \tag{6}$$

and will be convergent if the opposite is true. If, by hypothesis, $y = \xi(x)$, and equations (1) and (3) are, therefore, to be identical, the structure of the oscillating configuration must evidently be such that

$$\frac{apx^b + q}{x(ax^b - 1)} = \frac{4}{x} + \frac{d \log P}{dx} \tag{7}$$

and

$$\frac{arx^b + s}{x^3(ax^b - 1)} = \left\{\frac{\alpha}{x} - \frac{n^2 R}{\bar{\gamma}g}\right\} \frac{d \log P}{dx}, \tag{8}$$

respectively. Equation (7) implies that

$$\frac{d \log P}{dx} = \frac{a(p - 4)x^b + q + 4}{x(ax^b - 1)}, \tag{9}$$

which can be integrated into

$$P = k(1 - ax^b)^{(p + q)/b} x^{-(q + 4)}, \tag{10}$$

where k is a constant. Since the pressure must, by definition, vanish on the surface where $x = 1$, equation (10) makes it evident that this can be true if, and only if, $a = 1$. The first of the six arbitrary constants occurring in equation (3) has thus been specified.

Next let us eliminate the logarithmic derivative of P between (7) and (8) and solve the resulting equation for g: we obtain

$$g = \frac{x(Cx^b + D)}{Ax^b + B}, \tag{11}$$

where we have abbreviated

$$\left.\begin{array}{l} A = \bar{\gamma}[a(p - 4) - r] \\ B = \bar{\gamma}[a(q + 4) - s] \\ C = n^2 R(p - 4) \\ D = n^2 R(q + 4). \end{array}\right\} \tag{12}$$

Now, as is well known,

$$\varepsilon = G\,\frac{m(x)}{R^2 x^2},$$ (13)

where G denotes the constant of gravitation and $m(x)$, the mass of our configuration interior to x. Provided that the density is a continuous function of x,

$$m(x) = 4\pi GR^3 \int_0^x \rho x^2\, dx.$$ (14)

Inserting (11) in (14) and differentiating with respect to x we find that the density inside of our configuration should vary as

$$\rho(x) = \frac{3ACx^{2b} + [(3-b)AD + (3+b)BC]x^b + 3BD}{4\pi GR(Ax^b + B)^2}.$$ (15)

All constants in this equation are so far arbitrary—save for the obvious requirement that they be such as to render $\rho(x)$ positive throughout the interior. The central density of such a configuration is given by

$$\rho_c = \frac{3D}{4\pi GRB},$$ (16)

while its mean density becomes

$$\bar{\rho} = \frac{3}{4\pi GR}\,\frac{C+D}{A+B}.$$ (17)

Lastly, let us ensure that the configuration, in which the pressure, density, and gravity are governed by equations (10), (11) and (15), can be in hydrostatic equilibrium. As is well known, this will be the case provided that P, ρ and g are related by

$$\frac{dP}{dr} = \frac{1}{R}\frac{dP}{dx} = -g\rho.$$ (18)

If we differentiate (10) and insert in (18) together with (11) and (15), the equation of hydrostatic equilibrium will take the explicit form

$$\frac{4\pi k G}{3n^2 R}\frac{B^2}{D} = \frac{1 + \left[\left(1-\frac{b}{3}\right)\frac{A}{B} + \left(1+\frac{b}{3}\right)\frac{C}{D}\right]x^b + \frac{AC}{BD}x^{2b}}{x^{-(q+b)}(1-x^b)^{[(p+q)/b]-1}\left(1+\frac{A}{B}x^b\right)^3}$$ (19)

and must hold good throughout the interior (except, possibly, at the center). This latter equation supplies the remaining set of conditions

which the arbitrary constants in equation (3) must fulfill in order to provide a physically admissible solution. In what follows we shall, therefore, set out to ascertain all possible combinations of the values of b, p, q, r and s which conform to these conditions.

2. *Equilibrium Configurations.*—An inspection of equation (19) makes it evident that, if all four constants A, B, C, D as well as b are to be real and different from zero, this equation cannot be satisfied for any value of x; for if all four were non-vanishing, equation (19) would require that

$$\left.\begin{array}{l} (D/B)^2 = (4/3)\pi Gk(q+4) \\ q+6 = 0 \end{array}\right\} \tag{20}$$

be satisfied simultaneously, and this would render at least one of our constants imaginary. A closer examination discloses, moreover, that the same situation prevails if *one* of the four constants A, B, C, D is set equal to zero—except when $B = 0$ and $b = 3$, in which case Prasad's model follows. It is not until *pairs* of these constants are permitted to vanish simultaneously that we obtain all other configurations which are consistent with our initial assumption (2).

If $A = C = 0$ or $A = D = 0$, one of the two remaining constants still turns out to be imaginary. If $A = B = 0$, the corresponding configuration would be one of infinite mass, density and gravity; if $C = D = 0$, the mass of our configuration would be zero. The case of $B = D = 0$ leads to a homogeneous configuration (Sterne's "Model 1"), while if $B = C = 0$ two different types of configurations are possible. When $b = 2$, a model is obtained in which the density varies continuously as x^{-2}; the central density is infinite, but the mass of the whole configuration remains finite (Sterne's "Model 2"). If, however, $b = 3$, the density is discontinuous at $x = 0$ and such that the whole mass of the configuration will be stored at the center; the weight of the surrounding envelope being infinitesimal the gravity falls off with the inverse square of the distance from the center. A whole family of such models, characterized by $-4 < q < 0$, is found to be consistent with the assumption of hydrostatic equilibrium, but only one particular member of it (corresponding to $q = -1$) has so far been noticed (Sterne's "Model 3"). In what follows, all these models will be discussed in turn.

3. *Homogeneous Models.*—If, in equations (12), the values of our arbitrary constants q and s are chosen so as to render $B = D = 0$, the density of the respective configuration as defined by equation (15) turns out to be constant and equal to

$$\rho = \frac{3C}{4\pi GRA} = \frac{3n^2(p-4)}{4\pi G\bar{\gamma}[\bar{a}(p-4)-r]}, \tag{21}$$

and an appeal to equations (16) and (17) discloses that

$$\rho_c = \rho(x) = \bar{\rho}. \tag{22}$$

Now the condition $D = 0$ implies, by (12), that $q = -4$ and, consequently, $B = 0$ implies that $s = 0$. Moreover, equation (19) safeguarding the existence of hydrostatic equilibrium requires that $k = (2/3)\pi G\bar{\rho}^2 R^3$, $b = 2$ and $p = 6$. Inserting this latter in (21) we find ultimately that

$$r = \frac{3n^2}{2\pi G\bar{\rho}\bar{\gamma}} - 2. \tag{23}$$

Equations (10), (11) and (15) describing the model under investigation become

$$P = \frac{2}{3}G\bar{\rho}^2R^2(1 - x^2) \left.\begin{array}{l} \\ \\ \\ \end{array}\right\}$$
$$\rho = \bar{\rho} \tag{24}$$
$$g = \frac{4}{3}\pi G\bar{\rho}Rx.$$

If this model were disturbed slightly from its state of equilibrium in such a way that a small purely radial motion results, its amplitude $\xi(x)$ would be of the form (2) where the constants α, β and γ are obtained from equations (4) by inserting in them the foregoing values of p, q, r and $s;$ while equation (5) yields, for $q = -4$, $c = 0$ or -3. A requirement that there be no displacement at the center rules out the negative root; hence, $c = 0$. The outer boundary condition requiring that there be no variation of pressure on the surface can be met only if $\xi(1)$ is a finite quantity. Now the values of b, p and q in the present case turned out to be such that $p + q = b$, in which case the criterion (6) discloses that the hypergeometric series on the right-hand side will diverge for $x = 1$. If $\xi(1)$ is to be a finite quantity, we must reduce the respective infinite series to a polynomial by making α or β equal to zero or some negative integer by setting

$$r = -2j(2j + 5), \qquad j = 0, 1, 2, \ldots \tag{25}$$

which, combined with (21), specifies the characteristic frequencies n in which our configuration can oscillate freely in the jth mode. The corresponding amplitudes of oscillation then take the explicit forms

$$\xi_j(x) \propto G_j\left(\frac{5}{2}, \frac{5}{2}, x^2\right), \tag{26}$$

where $G_j(p, q, x) \equiv F(p + j, -j, q, x)$ denotes the respective Jacobi polynomial.[3]

The conditions $B = D = 0$ are not the only ones which lead to equation (21) for, as the reader can easily verify, a combination $B = 0$ and $b = 3$

will reduce (15) to exactly the same result. We thus obtain another homogeneous configuration which is, however, different from the preceding one; for the equation (19) of hydrostatic equilbrium now calls for $p = 6$ but $q = -3$, in virtue of which the condition $B = 0$ implies that $s = \bar{a}$, and

$$h = \frac{3n^4 R^2}{2\pi GA}\, r \tag{27}$$

where

$$A = \gamma(2\bar{a} - r). \tag{28}$$

On the other hand, equation (17) defining the mean density of our configuration takes the explicit form

$$\bar{\rho} = \frac{9n^2}{4\pi GA}. \tag{29}$$

Eliminating A between (27) and (28) by means of (29) we obtain

$$r = 2\bar{a} - \frac{9n^2}{4\pi G\gamma\bar{\rho}}, \tag{30}$$

and

$$h = \frac{8}{27}\, \pi GR^3 \bar{\rho}^2. \tag{31}$$

A comparison of equations (21) and (29) discloses that

$$\rho(x) = \frac{2}{3}\,\bar{\rho}, \tag{32}$$

while equation (10) assumes the explicit form

$$P = \frac{8}{37}\, \pi G\bar{\rho}^2 R^2 \frac{1 - x^3}{x}. \tag{33}$$

Both the preceding equations are different from (24) and the reason is not too hard to find. Unlike the preceding model, the validity of equation (21) must be limited to $0 < x < 1$, with the center $x = 0$ being a singular point. Our configuration must consist of a central mass-point, of mass m_0, surrounded by an envelope of constant density and mass m_e. The ratio of these two masses is already fixed by equation (32); for, by definition, we have

$$\frac{\rho(x)}{\bar{\rho}} = \frac{m_e}{m_0 + m_e} \tag{34}$$

which, combined with (32), yields

$$m_s = 2m_0. \tag{35}$$

Hence, the homogeneous envelope should contain two-thirds of the total mass of our configuration. The total gravity prevailing in our configuration will eventually be obtained from equation (11), the right-hand side of which is to be increased by the term Gm_0/R^2x^2 arising from the central mass; doing so we obtain

$$g(x) = \frac{m}{3R^2}\left\{2x + \frac{1}{x^2}\right\}, \tag{36}$$

where $m = m_0 + m_s$ denotes the total mass of our configuration.

If this model were disturbed slightly from a state of equilibrium in such a way that a small purely radial motion results, its amplitude would be of the form (2) where the constants α, β and γ are obtained by inserting in equations (4) the foregoing values of p, q, r and s, while the positive root of equation (5) is

$$c = \sqrt{1 + \bar{\alpha}} - 1. \tag{37}$$

As in the preceding case, we again have $\alpha + \beta = \gamma$ and the hypergeometric series $F(\alpha, \beta, \gamma, 1)$ again diverges. If ξ is to remain finite on the surface, therefore, the series must be terminated by the condition:

$$r = -(3j + c)(3j + c + 5), \tag{38}$$

where c is given by the preceding equation (37) and j is zero or a positive integer. Equation (38) combined with (30) specifies the characteristic frequencies n in which our configuration can freely oscillate in jth mode; the corresponding amplitudes take the explicit form

$$\xi_j(x) \propto x^c G_j\left(\frac{5}{3} + \frac{2}{3}c, \frac{5}{3} + \frac{2}{3}c, x^3\right) \tag{39}$$

where G_j denotes, as before, the respective Jacobi polynomial.

4. *Heterogeneous Models.*—The foregoing two homogeneous models characterized by $m_0/m_s = 0$ and 0.5, respectively, are the only types of configurations, consistent with our initial assumption (2), in which $\rho(x) =$ constant for $0 < x < 1$. In the remaining cases which yield a closed solution, the density will vary throughout the interior and our aim will be to choose this variation so as to make (2) a solution of our fundamental equation. Two kinds of such models will be found to exist: one exhibiting a finite degree of central condensation, the other consisting of a central point-mass surrounded by an envelope of infinitesimal weight and certain specific structure. This latter family of models will be found to possess

properties which are particularly interesting; but for the sake of completeness we shall take up the former and simpler model first.

This configuration is obtained by setting $B = C = 0$ or, which is the same, $p = 4$ and $s = 2\bar{\alpha}$. The equation (19) of hydrostatic equilibrium will then be satisfied if $b = 2$, $q = -2$ and

$$k = \frac{D^2}{8\pi G A^2} = \frac{2}{9} \pi G R^2 \bar{\rho}^2 \tag{40}$$

by (17). Moreover, equations (12) then yield $A = -r$ and $D = 2n^2 R$ which, combined with (17) gives

$$r = \frac{3n^2}{2\pi G \bar{\gamma} \bar{\rho}}. \tag{41}$$

Our configuration will be therefore characterized by

$$
\left.
\begin{aligned}
P &= \frac{2}{9} \pi G R^2 \bar{\rho}^2 \frac{1 - x^2}{x^2}, \\[2mm]
\rho &= \frac{\bar{\rho}}{3x^2}, \\[2mm]
g &= \frac{4\pi G R \bar{\rho}}{3x}.
\end{aligned}
\right\} \tag{42}
$$

Inside our model the density is found to vary as the inverse square of the distance from the center and to increase beyond any limit as $x \to 0$. This singularity is, however, integrable; for the mass of the configuration is clearly finite.

The constants α, β, γ characterizing the hypergeometric series on the right-hand side of (2) are obtained if we insert the above values of p, q, r and s in equations (4), while the positive root of (5) for $q = -2$ becomes

$$c = \frac{1}{2}\{\sqrt{1 + 8\bar{\alpha}} - 1\}. \tag{43}$$

The criterion (6) discloses that, for $p = 4$, $q = -2$, and $b = 2$, our hypergeometric series is again divergent where $x = 1$; hence, if the amplitude of the oscillations is to remain finite on the surface the series must be terminated by setting

$$r = (2j + c)(2j + c + 3), \tag{44}$$

where j is zero or a positive integer. The eigen-amplitudes (2) reduce then to Jacobi polynomials of the form

$$\xi_j(x) \propto x^c G_j\left(\frac{3}{2} + c, \frac{3}{2} + c, x^2\right), \tag{45}$$

while the characteristic frequencies of oscillation n are defined by the preceding equation (44).

In all models analyzed so far, the mass inside our configuration turned out to be a continuous and increasing function of x, with all layers contributing significantly to the effective gravity. In the final part of our discussion, a family of models will be considered in which the overwhelming part of the mass of the whole configuration is stored at its center so that, in effect, $m(x) = m(R)$ and the gravity inside of such a configuration varies, by definition, as

$$g = \frac{Gm(R)}{R^2 x^2}.$$ (46)

A comparison of this result with equation (11) discloses that if (46) is to be true, it is necessary that $B = C = 0$, i.e., $p = 4$, $s = \alpha(q + 4)$, $b = 3$ and

$$\frac{D}{A} = G \frac{m}{R^2} = \frac{4}{3} \pi G \bar{\rho} R.$$ (47)

Under these conditions we again have $A = -r$ which, combined with the preceding equation (47), yields

$$r = -\frac{3(q + 4)n^2}{4\pi G \bar{\gamma} \bar{\rho}}.$$ (48)

Moreover, by (10)

$$P = \lim_{k \to 0} k(1 - x^2)^{(q + 4)/3} x^{-(q + 4)}; \qquad x > 0.$$ (49)

Consistent with the assumption of the mass of the envelope surrounding the point-core to be infinitesimal, equation (15) yields indeed $\rho = 0$ (for $x \neq 0$). In order to ascertain the *variation* of this infinitesimal density within the envelope we must, therefore, fall back on the equation (18) of hydrostatic equilibrium which asserts that

$$\rho = -\frac{1}{g} \frac{dP}{dr} = \lim_{k \to 0} \frac{3(q + 4)k}{4\pi G R^2 \bar{\rho}} \frac{(1 - x^2)^{[(q+4)/3] - 1}}{x^{q + 3}}; \qquad x > 0.$$ (50)

Equations (49) and (50) render indeed the pressure and density infinitesimal everywhere except at the center where they must be, by definition, infinite.

In all preceding equations the value of q has so far been wholly arbitrary. The only restriction which we shall have to impose upon q will be a requirement that the total mass of the envelope be also a quantity of the order of k—i.e., that the integral on the right-hand side of (14) be convergent for $x = 1$. Explicitly,

$$m(R) = \frac{3(q+4)Rk}{G\beta} \cdot \int_0^1 (1 - x^3)^{[(4+q)/3]-1} x^{-(q+1)} \, dx$$

$$= \frac{(q+4)Rk}{G\beta} B\left(\frac{q+4}{3}, -\frac{q}{3}\right), \tag{51}$$

where B denotes the complete beta-function. The latter is known to be convergent if both its arguments are positive; and they will be such if

$$0 > q > -4. \tag{52}$$

The constants α, β and γ of the hypergeometric series in (2) expressing the amplitudes of our oscillation problem are again obtained when we insert the above values of p, q, r and s in equations (4). Equation (5) takes now the explicit form

$$c^2 - (q+1)c - \bar{\alpha}(q+4) = 0. \tag{53}$$

Its coefficients make it evident that, as long as $q > -4$, one of its two roots will be positive and equal to

$$c = \frac{q+1}{2} + \sqrt{\frac{(q+1)^2}{2} + \bar{\alpha}(q+4)}. \tag{54}$$

· The whole family of models corresponding to the range of q bounded by the inequality (53) can, for the purpose of description, be divided into three parts:

$$(a) \quad 0 > q > -1: \frac{q+4}{3} > 1; \quad \rho(1)/k = 0.$$

$$(b) \qquad q = -1: \frac{q+4}{3} = 1; \quad \rho(1)/k = \text{constant.}$$

$$(c) \quad -1 > q > 4: \frac{q+4}{3} < 1; \quad \rho(1)/k = \infty.$$

The criterion (6) discloses that if $0 > q > -1$ [which includes the models (a) and (b)] our hypergeometric series diverges when $x = 1$ and must, therefore, be terminated by putting

$$r = -\frac{3(q+4)n^2}{4\pi G\gamma\beta} = -(3j+c)(3j+c+3), \tag{55}$$

where c is given by (54) and j stands for zero or a positive integer. The eigen-amplitudes of our problem then take the form

$$\xi_i(x) \propto x^r G_i \left(1 + \frac{2}{3} c, 1 + \frac{2}{3} c, x^3 \right),$$ (56)

while the eigen-frequencies n are given by the foregoing equation (55). If $0 > q > -1$, equation (50) shows that the density becomes zero when $x = 1$; the reader may note that, of all models discussed so far, this is the only one in which the density as well as pressure vanish on the surface of our configuration. In the limiting case of $q = -1$ the density on the surface becomes arbitrary; this case was previously discovered by Sterne[1] (as his "Model 3").

If, however, q becomes less than -1, the criterion (6) discloses that the hypergeometric series will converge for an *arbitrary* value of r. The right-hand side of equation (2) automatically satisfies, therefore, our outer boundary condition requiring the finiteness of $\xi(x)$ on the surface without imposing any restriction on n—which means that *the corresponding configuration can perform free radial oscillations in any frequency.* This is the first instance of a "continuous spectrum" in the distribution of eigen-frequencies encountered in connection with any gaseous configuration in hydrostatic equilibrium known so far, and the properties of such configurations deserve evidently a close attention. Equation (50) discloses that their density $\rho(x)$, infinite at the center, becomes infinitesimal for $0 < x < 1$, but infinite again for $x = 1$, thus giving rise to an infinitesimally thin surface shell. The mass enclosed in this shell remains, however, a quantity of the order of k and does not, therefore, contribute appreciably to the effective gravity; our basic assumption which led us to (46) has not been violated. As long as $-1 > q > -3$, the density between the center and the surface shell diminishes at first with increasing x until the distance has been reached at which $2x^3 = q + 3$. At this point the density gradient reverses its sign and the density increases hereafter again until the surface is reached. Ultimately, if $-3 \geqslant q > -4$, the density gradient will be positive for $x > 0$ throughout the configuration.

5. *Concluding Remarks.*—Whether or not the physical situation analyzed in preceding paragraphs finds an application to actual astronomical bodies is an intriguing object for speculation. Our results may possibly have some bearing on the behavior of early-type giant or supergiant stars, in which the "surface shell" may be constituted by the condensing material driven out by the radiation pressure. Several theories of the structure of the giants along such lines were advanced in recent decades, the latest and most satisfactory one being due to Menzel.[6] If our configurations have anything to do with such stellar models, our results would imply that the models are hydrostatically stable under any small and spherically symmetrical disturbance, and would respond to it by setting up pulsations in an *arbitrary* period. For such stars, the product $P\sqrt{\bar{\rho}}$

(P being the pulsation period) could, therefore, assume *any* value.   Moreover, if the disturbing force were periodic, a resonance of the free and forced periods would be immediately established and the amplitude of the oscillation greatly enhanced.   Second-order effects, ignored in the present investigation, would thus probably soon become appreciable and could operate to destroy the resonance.   Should they fail, however, to do so, the only way in which a star built up according to our model could escape from its predicament would be through a gradual change of its whole internal structure which would convert it into another model characterized by a discrete spectrum of eigen-frequencies, or the old model might drift out of the region of continuous frequency spectrum.   Barring such an escape in time, however, the consequences of any periodic disturbance of the configurations built up according to our model might become conspicuous, or even cataclysmic—whatever the period of the disturbing force.   The extent to which any such phenomena may actually occur in Nature must, however, be left for future investigations to decide.

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[1] M. N., 97, 582 (1937).

[2] *Ibid.*, 108, 414 (1948).

[3] *Ap. J.*, 111, No. 1 (January, 1950); in press.

[4] Cf., for instance, ref. 1.   The equation was first derived by Eddington, *M. N.*, 79, 2 (1918).

[5] In the notations of Courant-Hilbert, *Methoden der Matematischen Physik*, Berlin, 1931, vol. I, p. 77.

[6] Zeeman Congress, Amsterdam (1946); *Physica*, 12, 768 (1946).

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THE SYNTHESIS OF RHODOPSIN FROM RETINENE1

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Rhodopsin, the light-sensitive pigment of rod vision, is bleached in the retina by light and is continuously resynthesized by ordinary "dark" reactions.   Its restoration permits vision to continue in the light, and is the source of visual dark adaptation.

Some seventy years ago Kühne observed that rhodopsin is synthesized in two ways—a rapid regeneration from yellow products of bleaching, persisting in the isolated retina and even to some degree in solution; and a much slower synthesis from colorless precursors, found ordinarily only in the intact eye, and requiring—so Kühne believed—the cooperation of substances from the pigment epithelium.[1]   These processes have since been

identified, respectively, as the synthesis of rhodopsin from retinene$_1$ and from vitamin A$_1$.[2]

Kühne's observation that rhodopsin is restored to some extent after bleaching in solution was confirmed by Hecht, *et al.*,[3] and Chase and Smith.[4] We can conclude from the way these contemporary experiments were performed that regeneration proceeded from the usual end-products of bleaching rhodopsin in solution, retinene$_1$ and protein. It was reported to follow the course of a first-order reaction, and to be optimal at pH 6.7. The largest regeneration recorded was about 15 per cent.

The bleaching of rhodopsin in solution is a complex process. An initial light reaction forms an orange-red product (lumi-rhodopsin) which is transformed by ordinary thermal reactions to a final mixture of retinene$_1$ and protein.[5] The conditions for the further reduction of retinene$_1$ to vitamin A$_1$—the presence of retinene reductase and reduced cozymase—are not realized in the solutions here considered.[6] As retinene$_1$ forms it is redistributed. In part it remains attached to the groups on rhodopsin-protein on which it originates, in part it couples with other groups on this and other molecules.[6, 7] This wandering of retinene$_1$ away from its original sites of attachment to rhodopsin-protein is an important factor limiting regeneration.

We have indeed good evidence that all stages in the bleaching of rhodopsin to retinene$_1$ are reversible; but that, the further bleaching has advanced, and the more time has been given for retinene$_1$ to leave rhodopsin-protein, the less regeneration occurs. Thus if the bleaching process is blocked in its initial stages (lumi-rhodopsin, meta-rhodopsin), as in extreme cold or dry gelatin films, regenerations of about 50 per cent are observed;[5] but after about an hour of illumination at room temperature in solution, only a few per cent of rhodopsin is regenerated.

These considerations led us to examine the effect of flooding rhodopsin solutions with synthetic retinene$_1$. The retinene$_1$ was prepared by the chromatographic oxidation of crystalline vitamin A$_1$ on solid manganese dioxide.[8] It was added in 2 per cent aqueous digitonin, the detergent used to extract rhodopsin from the retina. When rhodopsin is bleached in the presence of a high concentration of retinene$_1$, regenerations of about 70 per cent are regularly observed.

Such an experiment is shown in figure 1. A preparation of cattle rhodopsin was divided into three portions. To one a large excess of retinene$_1$ was added, and this and a second portion were exposed for one minute to intense white light. The extinction at 500 mμ—the absorption maximum of rhodopsin—was thereafter measured in darkness. In the solution flooded with retinene$_1$ the extinction rose rapidly as rhodopsin regenerated; in the untreated solution, after a small preliminary rise, the extinction fell due to secondary "dark" components of bleaching. When all changes were

nearly complete, hydroxylamine was added to these solutions and to the third portion of the original rhodopsin, to block any further regeneration (see below).[9] Absorption spectra were measured in the dark, then again after bleaching the three solutions in the light. The differences in absorption represent the rhodopsin present originally ($A - B$), and that regen-
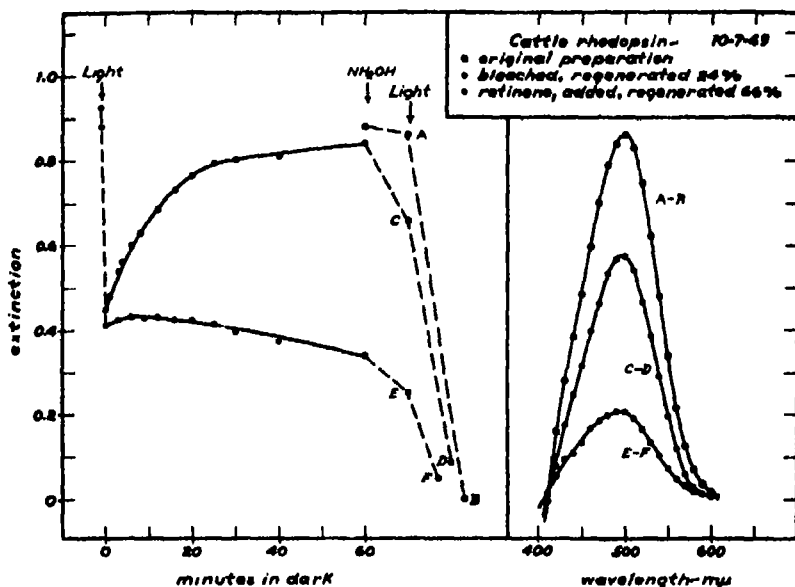
FIGURE 1

Regeneration of rhodopsin in the presence of added retinene₁.   25 °C., pH 6.2. A highly purified solution of cattle rhodopsin was divided into 3 portions. To one, a high concentration (ca. 30 μg. per ml.) of synthetic retinene₁ was added (solid circles); and this and a second untreated portion (open circles) were bleached in white light. Both were then left dark for 60 minutes. Measurements at the left show extinctions at 500 mμ, the wave-length of maximum absorption of rhodopsin. The solution to which retinene₁ was added shows a large rise in extinction at this wave-length, the other a net fall. Finally hydroxylamine (0.25 *M*) was added to these solutions and to the third portion of the original rhodopsin, to block further regeneration. Absorption spectra were measured in the dark; then all three solutions were bleached, and their absorption spectra remeasured. The differences in absorption, at 500 mμ on the left, and throughout the spectrum on the right, represent the rhodopsin present originally ($A - B$), and regenerated in the untreated solution ($E - F$) and in the solution to which retinene₁ had been added ($C - D$).

erated in the untreated solution ($E - F$) and in the solution to which retinene₁ had been added ($C - D$).

The retinene₁ added in such an experiment might act in a number of ways. It could tend to reverse the bleaching process in its intermediate

stages; or to retard the migration of retinene₁ away from rhodopsin-protein by occupying all other groups in the solution to which retinene₁ could attach. It probably does these things; but it also enters directly into the synthesis of new rhodopsin.

This was demonstrated in experiments in which colorless rhodopsin-protein, free of all native retinene₁, was mixed with synthetic retinene₁ in high concentration. This mixture, placed in the dark, yields a large synthesis of rhodopsin.
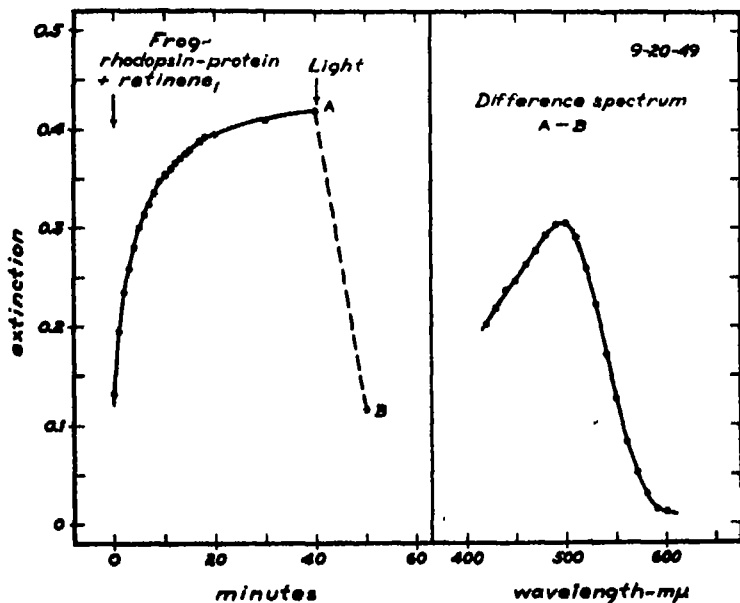
· FIGURE 2

Synthesis of rhodopsin from rhodopsin-protein and synthetic retinene₁. A colorless solution of rhodopsin-protein extracted from frog retinas which had bleached for 1¹/₂ hours in daylight was mixed with synthetic retinene₁ to a final concentration of about 10 μg. per ml. The measurements at the left show the rise in extinction in darkness at 500 mμ as rhodopsin is synthesized (25°C., pH 6.3). At *A* the product was exposed to daylight for 20 minutes; it bleached to *B*. The difference in absorption spectrum before and after bleaching (*A* − *B*) is shown at the right. It has the maximum at about 498 mμ characteristic of regenerated rhodopsin.

Such an experiment is shown in figure 2. Fifteen frog retinas (*Rana pipiens*) were isolated in dim red light, then left in white light for 1¹/₂ hours. At the end of this time they were colorless, the retinene₁ first formed in bleaching having been reduced to vitamin A₁. The retinas were homogenized and preextracted thoroughly with neutral phosphate buffer; this ex-

tract, flooded with retinene₁, yields no rhodopsin. Subsequent treatment with 2 per cent digitonin dissolves rhodopsin-protein, which even after losing its carotenoid prosthetic group requires a detergent for its extraction. The rhodopsin-protein was mixed with a digitonin solution of synthetic retinene₁, with the result shown at the left of the figure. The extinction at 500 mμ rose rapidly to a maximum value, attained in about 40 minutes at 25°C. On exposure to light this product bleached from *A* to *B*. The difference in absorption spectrum before and after bleaching ("difference spectrum") is shown at the right of the figure. It has the maximum at about 498 mμ characteristic of regenerated rhodopsin. The short-wave-length limb of this difference spectrum is abnormally high, due to some photodecomposition of retinene₁ in addition to the bleaching of rhodopsin. A more perfect difference spectrum obtained in a similar experiment is shown in figure 3 (curve 1).[10]

FIGURE 3

Synthesis of light-sensitive pigments from rhodopsin-protein mixed with retinene₁ and retinene₂. Rhodopsin-protein was extracted with digitonin from frog retinas which had been bleached to colorlessness in daylight. Portions of this were mixed with synthetic retinene₁ and retinene₂ in digitonin, and let stand in the dark at about 23°C. for 18 hours. The absorption spectra were measured in the dark, then again after bleaching in white light. The differences in the absorption spectra before and after bleaching are shown. That from rhodopsin-protein mixed with retinene₁ (1) shows the maximum at about 497 mμ characteristic of synthetic rhodopsin; that from the protein mixed with retinene₂ shows a maximum displaced to about 512 mμ.

Do other molecules than rhodopsin-protein and retinene₁ take part in this reaction? This question cannot yet be answered with certainty, yet all available evidence suggests that nothing more is needed. As one indication of this, the more highly purified our rhodopsin preparations, the better they regenerate. That shown in figure 1, for example, was made as follows. Cattle retinas were crushed, and the outer segments of the receptor cells

separated from the remaining retinal tissue by differential centrifugation. The outer limbs were tanned in 4 per cent alum, leached with water and neutral phosphate buffer, lyophilized and extracted twice in the dry state with petroleum ether. Finally rhodopsin was extracted with digitonin solution. Few other substances would have emerged from a procedure so narrowly adjusted to the peculiar properties of rhodopsin; and judging from its spectrum this was one of the purest solutions of this pigment yet prepared. Yet when mixed with highly purified retinene$_1$ it yielded an exemplary regeneration.

We have found that rhodopsin can be kept for many days, or dialyzed exhaustively against neutral phosphate buffer, without impairing its capacity to regenerate after bleaching. On the other hand we have not succeeded in increasing regeneration by adding various substances, among them adenosine triphosphate.

We conclude that in all probability this path for the synthesis of rhodopsin involves only the products of its bleaching. Rhodopsin-protein couples with retinene$_1$, and from it synthesizes its prosthetic group. This process probably involves the condensation of two molecules of retinene$_1$.[11] If one wishes to invoke an enzyme in this reaction, it should probably be rhodopsin-protein itself. Yet this view would violate a basic tenet of the enzyme concept, since rhodopsin-protein is removed stoichiometrically by the reaction.

Though these experiments do no more than make very probable that the synthesis of rhodopsin requires only retinene$_1$ and rhodopsin-protein, they make virtually certain that this is a spontaneous - i.e., an exergonic --- reaction. It does not have this appearance, since it is a rather complex synthesis. . But in fact, it is the bleaching of rhodopsin to retinene$_1$ and protein that requires energy, usually furnished by light. The reversion of retinene$_1$ and protein to rhodopsin is an energy-yielding reaction. This fact is of fundamental importance to an understanding of the visual cycle.

The regeneration of rhodopsin from rhodopsin-protein and retinene$_1$ is inhibited competitively by formaldehyde. One per cent formaldehyde causes an appreciable inhibition, 2-4 per cent almost complete blocking. We interpret this effect as a competition between formaldehyde and retinene$_1$ (vitamin A$_1$ aldehyde) for the amino groups on rhodopsin-protein with which both aldehydes readily couple. The regeneration of rhodopsin is also blocked completely by hydroxylamine (0.15 M), which "traps" retinene$_1$, presumably in the form of its oxime.

In every detail so far examined, the rhodopsin system has been closely mimicked by the porphyropsin cycle found in the rods of freshwater vertebrates. It has lately been shown, for example, that retinene reductase taken from either system reduces either retinene$_1$ or retinene$_2$ equally well to the corresponding vitamin A.[6]

Recently several attempts have been made to force the formation of porphyropsin in the eyes of rats[12] and persons[13] by feeding large quantities of vitamin A_2. These seem to have yielded positive effects, though small and still equivocal in meaning.

We have examined the effect of mixing frog rhodopsin-protein with a high concentration of synthetic retinene$_2$. This was made from a highly purified preparation of vitamin A_2[14] by chromatographic oxidation on manganese dioxide.[15] A synthesis of light-sensitive pigment occurs of about the same extent as with retinene$_1$. The difference in absorption spectrum before and after bleaching this product, however, is not characteristic of rhodopsin, but is displaced about 15 mμ toward the red, its maximum lying at about 512 mμ (Fig. 3). This brings it about half-way toward the position characteristic of porphyropsin. Whether we have to deal here with a mixture of photosensitive pigments, or with a modification of spectrum caused by the attachment of the porphyropsin prosthetic group to an abnormal protein, is still to be determined.

We have stressed in this paper the synthesis of photosensitive pigments from the retinenes, since this was accomplished by means employed in the retina. It should be noted, however, that we have in fact carried through for the first time the synthesis of rhodopsin from crystalline vitamin A_1, and the synthesis of a photopigment intermediate between rhodopsin and porphyropsin from pure vitamin A_2. The first step in each of these syntheses— the oxidation of the vitamins A to the corresponding retinenes—was accomplished, not as in the retina, but on manganese dioxide.

Yet we have already described an enzyme in the retina which could perform this oxidation also—retinene reductase, with cozymase (Coenzyme I, DPN) as its coenzyme. A number of comparable enzyme systems are known which reduce aldehydes to alcohols. All of them are potentially reversible, though always, as in this case also, their equilibria lie far over toward the side of reduction, the alcohol. To expose their reversibility it is common practice to use an aldehyde-fixing reagent—dimedone, semicarbazide, hydroxylamine, bisulfite—which by removing the aldehyde promotes a continuous displacement of equilibrium in the oxidative direction.[16]

It is obvious that the reaction described in the present paper, the spontaneous synthesis of rhodopsin, should constitute an efficient process for thus "fixing" retinene$_1$. Coupled with the retinene reductase system, this could drive a continuous though slow oxidation of vitamin A_1 to retinene$_1$, by continuously removing retinene$_1$ to form rhodopsin.

The difficulty with this system as it stands is that the *isolated* retina, though it contains retinene reductase, cozymase and rhodopsin-protein, forms only minimal amounts of rhodopsin from vitamin A_1. Either some additional factor makes this process operate more efficiently in the intact eye; or an alternative path exists for oxidizing vitamin A_1 to retinene$_1$ or to

rhodopsin. We have recently examined both the oxidation of vitamin A_1 to retinene$_1$, and the synthesis of rhodopsin from vitamin A_1, in retinal homogenates and extracts.[17] These experiments will be described in a subsequent paper.

Summary.—Cattle or frog rhodopsin, bleached in solution in the presence of high concentrations of synthetic retinene$_1$, regenerate about 70 per cent of their original content of rhodopsin when replaced in the dark. Rhodopsin is also synthesized *de novo* when its colorless protein moiety is mixed in solution with synthetic retinene$_1$. There is no evidence that other molecules participate in this reaction. It is clearly a spontaneous—i.e., an energy-yielding—process. It is inhibited competitively by formaldehyde, and also with such retinene-trapping reagents as hydroxylamine. When rhodopsin-protein is mixed in solution with synthetic retinene$_2$, a light-sensitive pigment is formed with its spectrum displaced in the direction of porphyropsin.

These experiments in fact accomplish the synthesis of rhodopsin from crystalline vitamin A_1, and of a comparable light-sensitive pigment from highly purified vitamin A_2. The vitamins A were oxidized to the corresponding retinenes, not by a retinal process, but by chromatographic oxidation on manganese dioxide. Nevertheless the pathways of these syntheses may bear a close relation to those which occur in the retina.

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² Wald, G., *J. Gen. Physiol.*, 19, 351 (1935–1936).

³ Hecht, S., Chase, A. M., Shlaer, S., and Haig, C., *Science*, 84, 331 (1936).

⁴ Chase, A. M., and Smith, E. L., *J. Gen. Physiol.*, 23, 21 (1939–1940).

⁵ Wald, G., Durell, J., and St. George, R. C. C., *Science* (in press) (1950).

⁶ Wald, G., *ibid.*, 109, 482 (1949).

⁷ Ball, S., Collins, F. D., Morton, R. A., and Stubbs, A. L., *Nature*, 161, 424 (1948).

⁸ Wald, G., *J. Gen. Physiol.*, 31, 489 (1947–1948). Based on a reaction discovered by Ball, S., Goodwin, T. W., and Morton, R. A., *Biochem. J.*, 42, 516 (1948).

⁹ The fall in extinction at 500 mμ on adding hydroxylamine to these solutions is caused by the fact that both the native and the added retinene$_1$ they contain is coupled with other molecules, in complexes which still display considerable absorption at 500 mμ. Hydroxylamine destroys these complexes by itself appropriating retinene$_1$, yielding presumably its oxime, with an absorption maximum at about 363 mμ, and almost no absorption at 500 mμ. Hydroxylamine was first used in this laboratory to block the regeneration of rhodopsin, in an experiment by Mr. Jack Durell.

¹⁰ Rhodopsin that has been regenerated or synthesized in solution has its spectrum at slightly lower wavelengths than rhodopsin extracted from the rods. This difference is apparent in the measurements of Chase and Smith,⁴ in figure 1 of the present paper, and in recent studies of the regeneration of rhodopsin from early stages of bleaching (Wald et al.⁵; Morton and Collins, Proc. 1st Internat. Congr. Biochemistry, Cambridge, 1949, p. 69). For this reason Morton and Collins have suggested that the rhodopsin regenerated in solution be called *iso*-rhodopsin. No other difference between it and retinal rhodopsin has yet appeared; nor is it yet clear that the regenerated

material is a single molecular species, rather than a mixture of the native and slightly modified pigments.

Note added in proof: Since this account was written we have observed regenerations of 85 per cent in purified cattle rhodopsin, flooded with retinene$_1$, and bleached for 1 minute in intense light.

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[12] Shantz, E. M., Embree, N. D., Hodge, H. C., and Wills, J. H., *J. Biol. Chem.*, 163, 455 (1946).

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[14] I am greatly indebted for this preparation to Dr. Edgar M. Shantz of the Research Laboratories of Distillation Products, Inc., of Rochester, New York.

[15] Wald, G., Meyerhof Festschrift, *Biochem. et Biophys. Acta*, 4, 215 (1950). Based on a reaction discovered by Morton, R. A., Salah, M. K., and Stubbs, A. L., *Biochem. J.*, 40, Proc. lix (1946).

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THE SYNTHESIS OF RHODOPSIN FROM VITAMIN A$_1$

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A number of years ago it was shown that in the rods of the retina, the light-sensitive pigment rhodopsin takes part in a cycle of the form:[1]
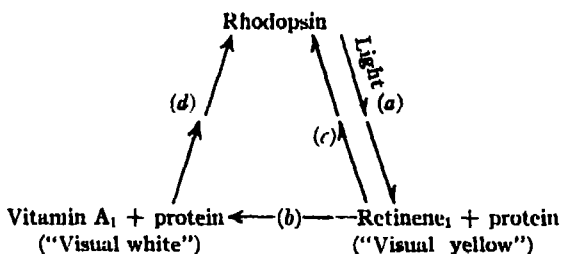
Some progress has been made recently with the chemistry of all these reactions. (*a*) In the bleaching of rhodopsin to retinene$_1$, the light reaction has been isolated and the orange intermediates, lumi- and meta-rhodopsin, characterized.[2] (*b*) Dihydrocozymase (DPN-H$_2$) has been identified as the coenzyme for the reduction of retinene$_1$ to vitamin A$_1$ by retinene reductase.[3] (*c*) Rhodopsin has been synthesized from rhodopsin-protein and retinene$_1$, the latter prepared by oxidizing crystalline vitamin A$_1$ with manganese dioxide.[4]

There remains reaction (*d*), the retinal synthesis of rhodopsin from vitamin A_1. We can identify this with the process which Kühne long ago called "neogenesis," describing it as the formation of rhodopsin from color-less precursors. It occurs appreciably only in the intact eye, and Kühne believed that it requires the cooperation of the pigment epithelium. Since he failed to see any evidence of orange or yellow intermediates in the course of this process, Kühne concluded that it does not retrace the path by which rhodopsin bleaches.[5] Indeed no direct evidence of a reversion of vitamin A_1 to retinene$_1$ can be found ordinarily in the isolated retina.

In the present paper, however, it is shown that isolated retinas, retinal homogenates and aqueous extracts of the retina can all form a little rhodopsin from vitamin A_1. When retinal homogenates are suitably supplemented, they synthesize almost half as much rhodopsin as is regenerated in the living eye during dark adaptation. It is shown also that one mechanism by which the retina can perform this synthesis—though perhaps not the only one—goes over retinene$_1$, and so retraces at least in part the path by which rhodopsin is bleached.

In the recent synthesis of rhodopsin from crystalline vitamin A_1 mentioned above, the first step—the oxidation of vitamin A_1 to retinene$_1$ (vitamin A_1 aldehyde)—was carried out, not by a retinal reaction, but by chromatographic oxidation on manganese dioxide. Retinene$_1$ prepared in this way condenses spontaneously with rhodopsin-protein to form rhodopsin.[4] The success of this procedure suggested that this might be a route for the synthesis of rhodopsin from vitamin A_1 in the retina.

The retina contains a potential mechanism for oxidizing vitamin A_1 to retinene$_1$ in the retinene reductase system. Under equilibrium conditions, this system primarily *reduces* retinene$_1$, transferring two hydrogen atoms to it from dihydro-cozymase:

$$C_{19}H_{27}CHO + DPN \cdot H_2 \quad \text{(Retinene reductase)} \quad C_{19}H_{27}CH_2OH + DPN$$

$$\text{Retinene}_1 \xrightarrow{\qquad\qquad\qquad\qquad} \text{Vitamin } A_1$$
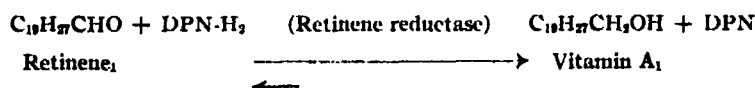
Similar DPN-enzyme systems in yeast and animal tissues reduce a variety of aldehydes to the corresponding alcohols.[6] All of them are potentially reversible, but always their equilibria lie far over toward the side of reduction. To demonstrate their reversibility it is common practice to "trap" the aldehyde as fast as it is formed, with one of a number of aldehyde-binding reagents. In this way the system is kept from coming to equilibrium, and is driven continuously in the oxidative direction.

By this means one can also oxidize vitamin A_1 to retinene$_1$ in a retinal preparation. To fix the aldehyde, we have used 0.1 M hydroxylamine, NH_2OH. This couples spontaneously with retinene$_1$ to form a pale yellow product—presumably retinene$_1$ oxime—with an absorption maximum at

about 355 mμ in hexane and 363 mμ in aqueous solution. The extinction of retinene₁ oxime at its maximum is about 1.4 times that of the free aldehyde.

Dark adapted frog retinas (*Rana pipiens*) were isolated, and were bleached in bright light to colorlessness. In this state all their rhodopsin had been converted to vitamin A₁ and protein. They were then homogenized, suspended in neutral phosphate buffer and incubated at room temperature. The untreated homogenate, like the intact retina, does not form observable amounts of retinene₁, nor does it do so on adding DPN. In the presence of hydroxylamine, however, the untreated homogenate oxidizes an appreciable fraction of its vitamin A₁ to retinene₁; and the yield is greatly increased on adding cozymase.

These observations are illustrated in table 1. A homogenate of 16 frog retinas was divided into 3 equal portions. One was extracted at once with hexane (*a*). The other portions were incubated for 2 hours, one with hydroxylamine (*b*), the other with both hydroxylamine and DPN (*c*), and

TABLE 1

Oxidation of Vitamin A₁ to Retinene₁ by a Frog Retinal Homogenate, Incubated for 2 Hours at 23 °C., with and without Hydroxylamine and DPN

The homogenate initially contained vitamin A₁ alone. The table shows the absorption maxima (λ_{max}) of hexane extracts of the homogenate, and the proportions of vitamin A₁ and retinene₁ which they contained. The homogenate was suspended in $M/15$ phosphate buffer, pH 6.84, and this buffer was also added in the amounts shown.

SAMPLE	RETINAL HOMOGENATE, ML.	BUFFER, ML.	HYDROXYL-AMINE (0.2 M), ML.	DPN, MG.	λ_{max}, Mμ	VITAMIN A₁ TRANSFORMED TO RETINENE₁, %
a	0.4	1.6	..	...	322	0
b	0.4	0.6	1.0	...	327	28
c	0.4	0.6	1.0	2.0	335	49

were then extracted with hexane. The absorption spectra of these extracts were measured. As shown in table 1, their absorption maxima (λ_{max}) shifted from the initial position characteristic of vitamin A₁ (*a*), about 5 mμ toward the red on incubation with hydroxylamine (*b*), and about 13 mμ toward the red on incubation with both this reagent and DPN (*c*). From this shift of spectrum it could be computed that in (*b*) the final mixture of retinene₁ and vitamin A₁ contained about 28 per cent retinene₁, in (*c*) about 49 per cent retinene₁.

Another experiment is shown in figure 1. Dark adapted retinas were bleached to colorlessness, homogenized with neutral phosphate buffer and 3 equal portions of homogenate were incubated at room temperature with hydroxylamine and DPN. In (*a*) the reaction was stopped at once by adding methyl alcohol to a concentration of 60 per cent; in (*b*) it was stopped after 1 hour, in (*c*) after 2 hours. All three preparations were ex-

tracted with hexane. The absorption spectra of these extracts are shown in the figure. Vitamin A_1 alone was present initially (a); about 38 per cent had been converted to retinene$_1$ in one hour (b); and about 51 per cent in two hours (c). The differences between the initial and later spectra (b − a, c − a) show that the absorption had risen in the region of the retinene$_1$ maximum (positive differences) and fallen in the region of the vitamin A_1 maximum (negative differences).

It is evident from these data that in the presence of an aldehyde-fixative, retinal preparations can oxidize vitamin A_1 to retinene$_1$. This process is aided by supplementation with DPN, the coenzyme of the retinene reductase system. The added DPN apparently replenishes the supply of coenzyme present in the retina initially, but rapidly destroyed by a nucleotidase after homogenization.[3]

The retina normally possesses a specific process which binds retinene$_1$, its condensation with rhodopsin-protein to form rhodopsin. Like all proper trapping reactions, there is an energy-yielding process. It is not nearly so efficient as the binding of retinene$_1$ by hydroxylamine, and is therefore nearly completely blocked in the presence of this reagent.[4] In the retina, however, rhodopsin-protein may substitute physiologically for hydroxylamine, and may drive a
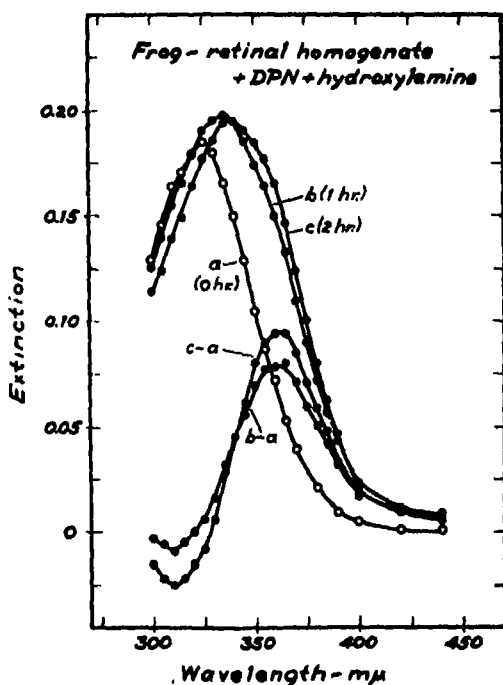
FIGURE 1

Oxidation of vitamin A_1 to retinene$_1$ in a homogenate of frog retinas containing 0.1 M hydroxylamine and 2 mg. DPN per ml. In (a) the reaction was stopped at once by adding methanol, in (b) after 1 hour of incubation at 23°C., in (c) after 2 hours incubation. pH 6.8. Absorption spectra of hexane extracts of these preparations are shown (a, b, c). The initial spectrum (a) is that of vitamin A_1; but this is displaced toward the red as the reaction proceeds. The changes in absorption (b − a, c − a) show a rise in the region of the retinene$_1$ maximum (positive differences), a fall in the region of the vitamin A_1 maximum (negative differences). From these data it is computed that 38 per cent of the vitamin A_1 initially present had been oxidized to retinene$_1$ in 1 hour, 51 per cent in 2 hours.

continuous oxidation of vitamin A_1 to retinene$_1$, by removing retinene$_1$ to form rhodopsin.

The difficulty with this idea is that the isolated retina, bleached to color-lessness, does not regenerate rhodopsin efficiently from vitamin A_1, though it contains all the components of the system described above—retinene reductase, cozymase and rhodopsin-protein. It is primarily this behavior of the isolated retina that caused Kühne and later workers to conclude that the synthesis of rhodopsin from colorless precursors requires new materials, and involves something more than the simple reversal of bleaching. This inadequacy of the isolated retina constitutes the primary problem with which our further experiments are concerned.

Frog retinas, isolated in the dark adapted condition, and bleached in the light to colorlessness, contain vitamin A_1 as their only carotenoid. If they are replaced in the dark for several hours, they still appear colorless, or at most faint pink. If, after incubation in the dark, they are extracted with 2 per cent aqueous digitonin, the extract is found to contain a small amount of rhodopsin. This is conveniently revealed by measuring the absorption spectrum of the extract in darkness, then again after bleaching in the light. The difference in absorption spectrum before and after bleaching—the so-called "difference spectrum"—is characteristic of rhodopsin.

The yield of rhodopsin in such an experiment can be estimated by ex-tracting dark adapted frog retinas with digitonin by the same procedure. The difference spectra of such extracts show that on the average each retina contributes an amount of rhodopsin having an extinction at 500 mμ of about 0.08 in 1 ml. of extract, measured in a layer 1 cm. in depth.

On this basis the intact bleached retina replaced in the dark regenerates from vitamin A_1 about 10 per cent of its potential content of rhodopsin. The yield is not increased by adding DPN. This in itself is not significant, since DPN probably does not penetrate the intact tissue.

A homogenate of bleached retinas regenerates about as much rhodopsin in the dark as do intact retinas. When DPN is added to such a homogenate, however, the yield of rhodopsin is approximately doubled.

Such an experiment is shown in figure 2. Sixteen retinas had been bleached in bright light for 45 minutes, and were wholly colorless. They were homogenized with neutral phosphate buffer, and divided into three equal portions. The first of these, extracted at once with hexane, showed the presence of vitamin A_1 alone (A). The other two portions were incu-bated in the dark at room temperature for 9 hours, one untreated (B), the other with DPN added (C). They were then extracted with digitonin, and the spectra of the extracts measured before and after bleaching. These difference spectra are shown at the left in figure 2. The solid residues from these extractions were reextracted with hexane; the spectra of these ex-tracts are shown at the right.

The hexane extracts show that vitamin A_1 alone is present, both in the original homogenate and in the portions incubated in darkness. The difference spectra at the left are characteristic of rhodopsin. They show that the untreated homogenate regenerated about 10 per cent of rhodopsin, while the homogenate incubated with DPN yielded almost twice this amount, about 18 per cent (cf. table 2).
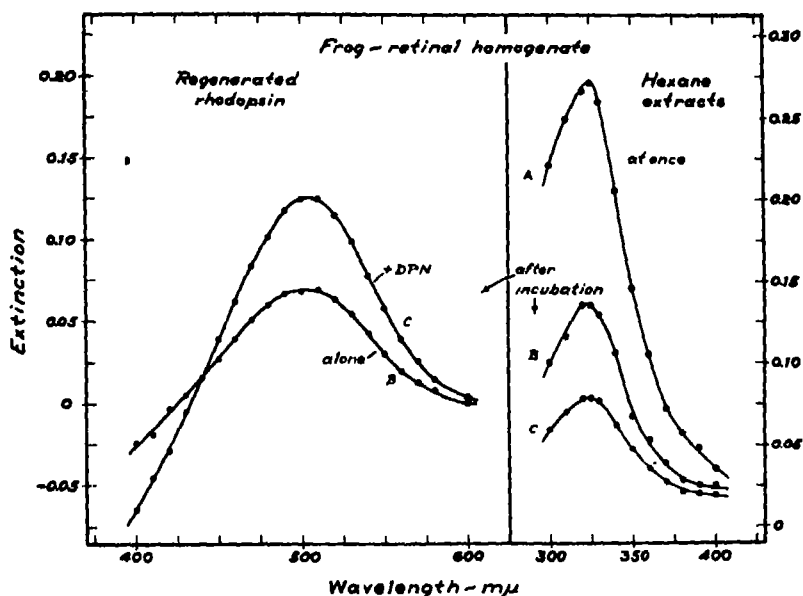
FIGURE 2

Synthesis of rhodopsin from vitamin A_1 in a retinal homogenate, incubated in the dark 9 hours at 23°C., with and without added cozymase (DPN, 4 mg. per ml.). The data at the left show difference spectra of digitonin extracts of these preparations -their differences in absorption before and after exposure to light. They are characteristic of rhodopsin, and show that supplementation with DPN approximately doubles the yield of this pigment. The absorption spectra at the right are of hexane extracts (A) of the original homogenate, and (B) and (C) of the residues of the incubated homogenate after extraction with digitonin. They show that vitamin A_1 is the only carotenoid present initially and at the end of the reaction.

The effectiveness of adding DPN in this synthesis suggests that it was accomplished through the oxidation of vitamin A_1 to retinene, by the retinene reductase system, retinene, being trapped by rhodopsin-protein as rapidly as formed. The data show that in the course of this process no free retinene, accumulates. Kühne's failure to see yellow intermediates during the "neogenesis" of rhodopsin is thus confirmed. Yet this is no assurance that retinene, is not an intermediate in the reaction.

We have already noted Kühne's insistence upon the co-operation of the pigment epithelium in "neogenesis." We find that when a homogenate of the pigment layers of the eye—pigment epithelium and choroid—is added to a retinal homogenate, the synthesis of rhodopsin from vitamin A_1 is approximately doubled. When both DPN and pigment layers are added to the retinal homogenate, the yield of rhodopsin is again doubled; it now approaches 40 per cent.

Such an experiment is shown in figure 3. Sixteen frog retinas were isolated in the dark, and were bleached to colorlessness in the light for 1 hour. They were homogenized in neutral phosphate buffer and the homogenate was divided into 4 equal portions. To one of these, the homogenate of the pigment layers from 12 eyes was added together with 4 mg. of DPN (*d*). To two other portions, 2 and 4 mg. of DPN alone were added (*b, c*). The

TABLE 2

PER CENT REGENERATION OF RHODOPSIN IN RETINAL HOMOGENATES, INCUBATED IN THE DARK FOR 8–9 HOURS UNDER VARIOUS CONDITIONS.

All homogenates were suspended in neutral phosphate buffer, final pH about 6.8. 23 °C. The per cent regeneration represents the extinction at 500 mμ of the regenerated rhodopsin as a fraction of the average extinction at 500 mμ of rhodopsin from an equal number of dark adapted retinas.

EXPERIMENT		DESCRIPTION	PER CENT REGENERATION
I. (Fig. 2)	(*a*)	Homogenate alone	10.0
	(*b*)	Homogenate + DPN	18.3
II. (Fig. 3)	(*a*)	Homogenate + DPN	17.9
	(*b*)	Homogenate + DPN + pigment layer homogenate	30.3
III.		Homogenate + DPN	25.4
IV.	(*a*)	Homogenate + pigment layer homogenate	20.3
	(*b*)	Homogenate + pigment layer homogenate + DPN	42.0

fourth portion (*a*) was extracted immediately with hexane; so also was a control portion of pigment layers (*e*). Homogenates *b, c* and *d* were incubated for 8 hours in the dark, and were then extracted with digitonin solution. The difference spectra of these extracts are shown at the left of figure 3. After extraction with digitonin, the solid residues were reextracted with hexane; the absorption spectra of these and the control hexane extracts are shown at the right of the figure.

It is clear from figure 3 that the addition of 2 mg. of DPN to a total of 0.7 ml. of reaction mixture produces a maximal effect on the regeneration of rhodopsin (*b*). Doubling this amount does not improve the yield further (*c*). On the other hand the addition of both DPN and homogenized pigment layers approximately doubles the yield of rhodopsin (*d*) (cf. table 2). The pigment epithelium therefore must add something other than DPN to the reacting system.

The spectra of carotenoid extracts at the right of figure 3 show that vitamin A_1 alone is present in the retinal homogenates, vitamin A_1 and xanthophyll in the homogenates containing pigment layers. In no case is any retinene$_1$ apparent, either in the tissues extracted at once or in those incubated in darkness.
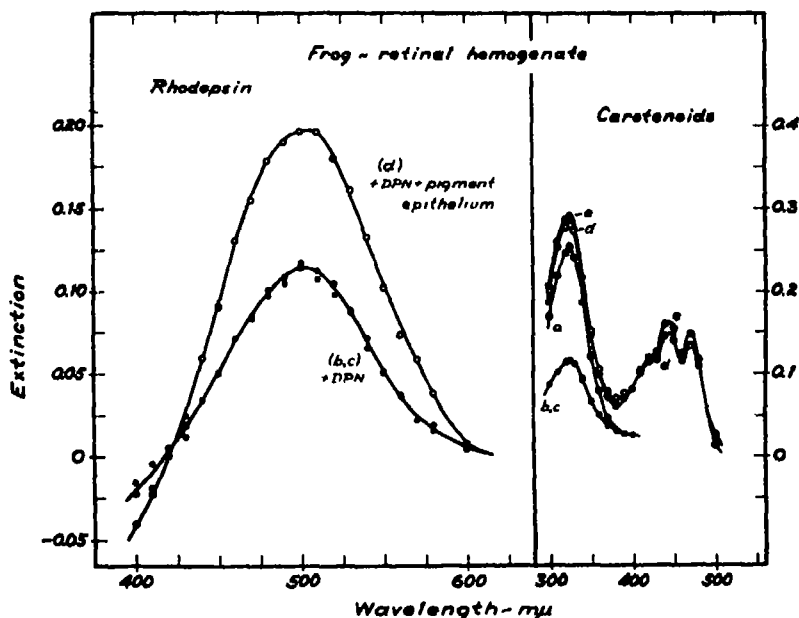
FIGURE 3

Synthesis of rhodopsin from vitamin A_1 in a retinal homogenate, incubated in the dark 8 hours at 23 °C., with (b) 2 mg. and (c) 4 mg. DPN added; and with (d) 4 mg. DPN and pigment layer homogenate added. The data at the left show the difference spectra of digitonin extracts of these preparations. They are characteristic of rhodopsin, and show that supplementation with 2 mg. DPN is as effective as 4 mg. DPN (b, c); but that the addition of pigment layer homogenate approximately doubles the yield of rhodopsin. The absorption spectra at the right are of hexane extracts of the solid residues of these preparations, and of control portions of the original retinal homogenate (a) and of the pigment layer homogenate (e). They show that the retinal preparations contain vitamin A_1, the pigment layer preparations vitamin A_1 and xanthophyll as their only carotenoids at the beginning and end of the reaction. Vitamin A_1 is responsible for the single absorption band in the ultra-violet at about 325 mμ, xanthophyll for the complex spectrum in the visible region.

The yields of rhodopsin obtained in retinal homogenates by all the types of treatment described above are summarized in table 2. We have, however, also observed the synthesis of rhodopsin from vitamin A_1 in aqueous solution.

An aqueous digitonin extract of bleached frog retinas contains rhodopsin-protein and retinene reductase. If freshly prepared, it also contains cozymase, though this is destroyed within a few hours by the nucleotidase which is present.[3] The addition of a high concentration of vitamin A_1 to such an extract might be expected to force the production of some retinene₁, and from this could induce the synthesis of some rhodopsin.
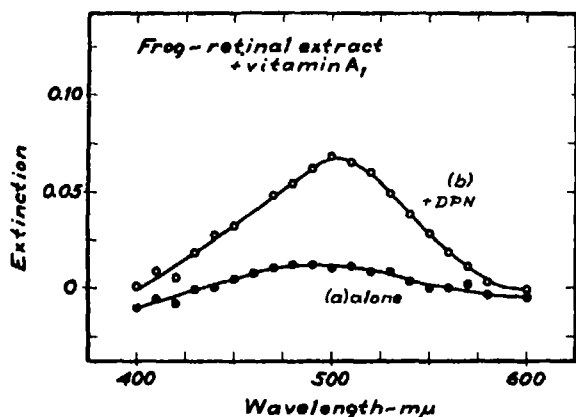
FIGURE 4

Synthesis of rhodopsin from vitamin A_1 in an aqueous extract of frog retinas. The retinas had been bleached to colorlessness, extracted with 2 per cent aqueous digitonin, and to this crystalline vitamin A_1 in digitonin was added. Half of this preparation was incubated in the dark overnight at 23°C. without further treatment (*a*), the other half with 4 mg. DPN added per ml. of extract (*b*). The data show the difference spectra of the final solutions. The solution without added DPN had synthesized a scarcely perceptible amount of rhodopsin; that supplemented with DPN had regenerated about 10 per cent as much rhodopsin as was present originally in the dark adapted retinas from which it was prepared.

Such an experiment is shown in figure 4. Twelve dark adapted frog retinas were isolated, bleached to colorlessness, homogenized and the homogenate extracted for 3 hours with digitonin. The extract was cleared by centrifuging at high speed, and to it a clear solution of crystalline vitamin A_1 in digitonin was added. The mixture was divided into halves, and to one portion 3.5 mg. of DPN was added. Both portions were left in the dark for 12 hours, and then their difference spectra were measured. These are shown in the figure. The solution to which no DPN had been added (*a*) regenerated an almost negligible amount of rhodopsin. That supplemented with DPN (*b*) had regenerated about 10 per cent as much rhodopsin as was

present originally in the dark adapted retinas from which the solution was prepared.

In summary, therefore, we have found that rhodopsin is synthesized from vitamin A_1 in intact retinas and retinal homogenates, with yields of about 10 per cent. In retinal homogenates supplemented with either DPN or pigment layer homogenate, the yields are about 20 per cent; and in homogenates supplemented with both DPN and pigment layers they rise to about 40 per cent. Aqueous extracts of retina, to which DPN and vitamin A_1 have been added, also synthesize rhodopsin with a yield of about 10 per cent.

The mechanism of rhodopsin synthesis in these preparations is still uncertain. We know one possible mechanism, the oxidation of vitamin A_1 to retinene$_1$ by the retinene reductase system, coupled with the condensation of retinene$_1$ with rhodopsin-protein to form rhodopsin. Yet this apparatus, as we find it in the isolated retina, appears unable to account for more than a small fraction of the rhodopsin formed in the intact eye.

It seems reasonably clear that DPN stimulates the synthesis of rhodopsin in our preparations by supplying coenzyme to the retinene reductase system. Pigment layer homogenate may act upon the same system, by supplying respiratory factors which drive it in the oxidative direction. In this regard it is perhaps significant that riboflavine, the common oxidant of DPN-H_2 in cellular respiration, is present in high concentration in the pigment epithelium, though little is found in the retina.[7]

We think it almost certain that some rhodopsin is synthesized through the intermediate oxidation of vitamin A_1 to retinene$_1$ by retinene reductase. It is conceivable that by coupling with respiratory mechanisms, the efficiency of the retinene reductase system is so greatly increased that it can account for the whole synthesis of rhodopsin from vitamin A_1. On the other hand alternative pathways for this process may exist, and may even be of major importance. These problems are being investigated further.

Summary.—Intact frog retinas and retinal homogenates can synthesize rhodopsin from vitamin A_1 in amounts about 10 per cent as great as are formed during dark adaptation *in vivo*. When either cozymase (DPN) or a homogenate of the pigment layers of the eye—pigment epithelium and choroid—are added to a retinal homogenate, the yield of rhodopsin is approximately doubled; and by the addition of both DPN and pigment layer homogenate, the yield is doubled again, bringing it to about 40 per cent. Aqueous extracts of retina, supplemented with DPN and vitamin A_1, also synthesize rhodopsin with a yield of about 10 per cent.

The mechanism of this synthesis is not yet wholly understood. Retinal homogenates slowly oxidize vitamin A_1 to retinene$_1$ (vitamin A_1 aldehyde) in the presence of such an aldehyde-binding reagent as hydroxylamine. This process, like rhodopsin synthesis, is aided by supplementation with

DPN, the coenzyme of the retinene reductase system. Hydroxylamine apparently drives the retinene reductase system in the oxidative direction, by removing retinene₁ as fast as it is formed.

In the retina, rhodopsin-protein may act similarly, since it is known to bind retinene₁ by condensing with it spontaneously to form rhodopsin. This is almost surely one mechanism for the synthesis of rhodopsin from vitamin A_1. Yet the isolated retina, which contains all the components of this system, regenerates very little rhodopsin from vitamin A_1. Either the retinene reductase system operates much more efficiently in the whole eye than in the isolated retina, through the action of such auxiliary factors as may be added by the pigment epithelium; or some rhodopsin is synthesized from vitamin A_1 by alternative mechanisms still to be explored.

[1] Wald, G., *J. Gen. Physiol.*, 19, 351, 781 (1935–1936).

[2] Wald, G., Durell, J., and St. George, R. C. C., *Science*, in press (1950).

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ANTIBIOTIC SUBSTANCES FROM BASIDIOMYCETES. VI. AGROCYBE DURA*

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In a previous report[1] from this laboratory, *Agrocybe dura* (L386.10)[2] was found to evidence considerable activity against *Staphylococcus aureus* (H) and *Escherichia coli* when tested by the streak of disk methods. Culture liquids of this fungus were found to have some activity. Further investigation has resulted in additional information on the antibiotic properties of *Agrocybe dura*.

Antibiotic Material in Liquid Culture.—Culture liquids with antibacterial activity were produced by growing the fungus at 25°C. in 2800 ml. Fernbach flasks containing a corn steep medium, as previously described.[3]

The fungus grew with medium rapidity; it required from 6 to 8 weeks to produce culture liquids of from 256 to 512 dilution units per ml. against *Staph. aureus*. When the active culture liquid was decanted and replaced with a liter of fresh corn steep medium, an activity of 256 dilution units per ml. against *Staph. aureus* developed in about two weeks.

A sample of culture liquid was Seitz filtered at its natural pH of 4.6, at pH 3.0, and at pH 7.0. More than three-fourths of the activity was removed by the filtration at pH 3.0; about one-half at pH 4.6; and none at pH 7.0. Adjusting to pH 3.0 and then neutralizing did not destroy the active substance. The filtered solutions of pH 4.6 and pH 7.0 were placed at 11°C. for 26 days with assay at frequent intervals. Neither solution lost activity.

Concentration of Active Material.—Preliminary experiments showed that culture liquid dried in the air lost about one-half of its activity. Because of this observation, which was confirmed by later experiments, care was used to avoid drying at any stage in the concentration of the active material.

Aqueous concentrates of antibacterial material were prepared by extracting the culture liquid with methyl-iso-butyl ketone. The solvent was evaporated to small volume *in vacuo*. The residue in the still was dissolved in alcohol which was diluted with water or the residue was dissolved in ether which was then washed with bicarbonate solution as described later and evaporated over a layer of water. Such concentrates had an activity of 1,000,000 or more dilution units per mg. against *Staph. aureus*.

Isolation of Crystalline Material.—A crystalline antibiotic substance was isolated from the culture liquid. The procedure for one lot was as follows: A volume of 10.5 liters of culture liquid with an activity of 128 dilution units per ml. against *Staph. aureus* was extracted twice with 750 ml. of methyl-iso-butyl-ketone; the ketone was evaporated to very small volume *in vacuo;* and ether added. The ether solution was extracted with 2% sodium bicarbonate solution to remove acids and part of the colored substances. Brown colored crystals were obtained upon evaporation of the ether at room temperature. These crystals were purified by recrystallization from 20% alcohol or from ether.

Characteristics of Crystalline Material.—The white crystals obtained were stable in air for about one day after which they turned black and became insoluble in ether. The crystals did not change in color when kept at 4°C. *in vacuo*. On attempting a melting point in air, the crystals turned black and then exploded at a temperature of about 145°. In a capillary *in vacuo*, the crystals decomposed at about 90° and evolved gas at 140°C. An analysis[4] indicated 65.58% C and 4.31% H. We propose to name this substance agrocybin.

Agrocybin was soluble in alcohol, acetone, ether, chloroform, methyl-

iso-butyl-ketone, slightly soluble in water and insoluble in hexane. The optical rotation of agrocybin in alcohol was zero for the sodium D line. The ultra-violet absorption spectrum of agrocybin in alcohol had peaks at 216, 224, 269, 286, 304 and 325 mμ. The curve obtained in pH 6.0 phosphate buffer was nearly the same as that obtained in alcoholic solution. The curve, figure 1, resembled greatly that of nemotin A.[5] There were sufficient differences, however, to indicate that the two substances were not the same.

Agrocybin was inactivated by heating to 100° at pH 8.5 and higher but not at pH 6.5. It was not inactivated in one hour at 25° and pH between 3.0 and 11.0. It did not distill with steam. Agrocybin was not extracted from ether by aqueous solution of pH 8.5 and probably is a neutral or a very weakly acidic substance.

FIGURE 1

Absorption spectrum of crystalline agrocybin in alcohol. The optical density is given for solutions measured in a 1 cm. cuvette. The dashed line refers to a concentration of 1 microgram per milliliter, and the continuous line to a concentration of 10 micrograms per milliliter.

Evidence for Existence of Agrocybin in an Inactive Form.— Part of the activity of a culture liquid was in an inactive ketone-insoluble form which was made active and ketone-soluble by boiling the solution.[6] In this respect the behavior of the culture liquid of *Agrocybe dura* was similar to that found previously[7] for the culture liquid of *Polyporus biformis*. A concentrate of the active substance free in the culture solution and a concentrate prepared from a culture solution heated after the free substance had been removed had the same antibacterial and antiluminescent activities. It is probable that the antibacterial substance formed by heating the culture liquid was the same as that which occurred free in the liquid. Since the antibacterial titer of a culture liquid was doubled by heating there appeared to be as much of the active substance in the inactive form as there was free.

Antibacterial and Antiluminescent Activity.—The antibacterial and antiluminescent activites of agrocybin were obtained by the methods in use in this laboratory[8] with the results set forth below. The activity is expressed as the minimum inhibitory concentration in micrograms per ml.

BACTERIA	ACTIVITY	BACTERIA	ACTIVITY
Bacillus mycoides	0.5	*Mycobacterium smegma*	1
Bacillus subtilis	0.5	*Photobacterium fischeri*	0.125
Escherichia coli	1	*Pseudomonas aeruginosa*	0.5
Klebsiella pneumoniae	1	*Staphylococcus aureus* (H)	1
Mycobacterium phlei	0.5		

The antiluminescent activity against *Photobacterium fisheri* is given in the following table:

TIME OF INCUBATION	ACTIVITY
10 min.	0.016
1 hrs.	0.008
2 "	0.004
3 "	0 002
24 "	0.064

Agrocybin belongs in the group of substances inhibitory for gram-positive, gram-negative and acid-fast bacteria. The antibacterial activities resemble those of biformin except that agrocybin is much more active than biformin against *B. mycoides* and *P. aeruginosa*. Although there may be a close chemical relation between agrocybin and nemotin A, as indicated by similar ultra-violet spectra, agrocybin is much more active against the gram-negative bacteria than is nemotin A.

The antiluminescent activity of agrocybin is much higher than that of any other substance tested and is about 60 times as great as the best preparation of biformin,[7] the second most active substance investigated.

Antifungal Activity. The activity of two concentrates prepared from the culture liquid was measured against nine fungi by serial dilution[9] and by an agar disk method. The fungi were: *Aspergillus niger, Chaetomium globosum* (USDA 1042.4), *Gliomastix convoluta* (PQMD4c), *Memnoniella echinata* (PQMD1c), *Myrothecium verrucaria* (USDA 1334.2), *Penicillum notatum* (832), *Phycomyces Blakesleeanus* (+ strain), *Stemphylium consortiale* (PQMD41b) *and Trichophyton mentagrophytes.*

When measured by the serial dilution method, the concentrates were about one-eighth to one-fourth as active against the fungi as against *Staph. aureus* except for *P. Blakesleeanus* and *G. convoluta* which were 32 and 128 times respectively as resistant as *Staph. aureus.*

Activity in the Presence of Blood.—Agrocybin and the antibacterial material in the culture liquid and in concentrates were inactivated to a considerable degree by rabbit and by human blood. The activity of agrocybin against *Staph. aureus* was reduced to $1/_{32}$ by incubation for 3 hours in a beef extract medium containing 5% whole human blood. A ketone concentrate was not reduced in activity by incubation with 5% human plasma and lost only one-half of its activity when incubated with 5% washed or hemolyzed human red cells.

Animal Toxicity.—A solution of 1 mg. per ml. of agrocybin was prepared in 0.7% sodium chloride solution and quantities ranging from 0.1 ml. to 1 ml. were injected into a tail vein of 16–18 g. Carworth Farms CFI male white mice. The larger the dose, the sooner the mice died. The animals receiving 1 mg. of agrocybin died within 1 hour, those receiving 0.1 mg. died in about 18 hours. The LD_{50} was less than 6 mg. per kg. body weight of white mice. Agrocybin caused a dermatitis in a human subject susceptible to bioformin.

Because of its high toxicity and inactivation by blood, experiments on possible therapeutic action were not attempted.

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QUANTITATIVE EFFECTS OF PROTEIN ENRICHMENT OF DIET UPON GROWTH AND EARLY ADULT LIFE*

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Previous publications have shown that with experimental animals (rats) eating *ad libitum* of a basal diet, adequate for their normal nutrition but of relatively low calcium content (0.18 to 0.2 per cent of the air-dry food mixture) the weight of the body may grow more rapidly than does the weight of calcium which the body contains. In such cases, notwithstanding continuously positive calcium balances, there may develop a low-calcium condition of body, shown by a dip in the curve which represents the *percentage* of calcium in the growing body.[1]

Moreover, among subjects of the same age and sex, living on the same

TABLE 1

FINAL AVERAGE RECORDS FOR RATS FED A WHEAT-AND-MILK BASAL DIET, ALONE AND WITH ADDED PROTEIN AS POULTRY MEAT

(10 Rats of Each Sex on Each Point, Full Litter-Mate Control)

	ON DIET 16 (14% PROTEIN): BASAL DIET OF WHEAT AND MILK — MEAN WITH ITS P.E.	C.V.	ON DIET 16 P 5 (24% PROTEIN): BASAL DIET + MEAT — MEAN WITH ITS P.E.	C.V.	DIFFERENCE WITH ITS P.E.	CRITICAL RATIO
Gain in weight, 28–56 days of age						
Females, g.	39.7 ± 1.59	19	61.6 ± 2.05	16	21.9 ± 2.59	8.5
Males, g.	48.5 ± 2.71	26	81.5 ± 2.51	14	33.0 ± 3.69	8.9
Age of females at birth of first young, days	132 ± 6.3	22	98 ± 3.0	14	34 ± 7.0	4.9
Young borne per female	41 ± 3.7	42	44 ± 4.0	43	3 ± 5.4	0.6
Young reared per female	29 ± 2.5	41	35 ± 3.5	47	6 ± 4.3	1.4
Average weight of young at 28 days of age, g.	40.5 ± 0.33	21	45.3 ± 0.32	20	4.8 ± 0.46	10.4
Length of reproductive life of females, days	368 ± 31.7	40	349 ± 20.5	28	19 ± 38	0.5
Reproductive life as per cent of life cycle, %	44 ± 3.9	42	47 ± 2.3	23	3 ± 4.6	0 7
Length of life						
Females, days	848 ± 22.3	12	764 ± 40.5 •	25	84 ± 46	1.8
Males, days	741 ± 21.0	13	748 ± 23.4	15	7 ± 31	0.2

TABLE 2

ALL AVAILABLE CASES FROM DIET 16 P 5 AND MOST CLOSELY RELATED CONTROLS FROM DIET 16

	DIET 16 — MEAN WITH ITS P.E.	C.V.	DIET 16 P 5 — MEAN WITH ITS P.E.	C.V.	DIFFERENCE WITH ITS P.E. FROM DIET 16	CRITICAL RATIO
Females:						
Gain in weight, 28–56 days, g.	(26)* 42 ± 1.2	22	(28) 61 ± 1.3	17	19 ± 1.7	11.2
Age at birth of first young, days	(26) 122 ± 3.33	21	(28) 106 ± 2.85	21	16 ± 4.4	3.6
Weight at 100 days, g.	(12)† 141 ± 2.77	10	(20) 168 ± 2.32	9	27 ± 3.6	7.5
Weight at 210 days, g.	(14) 189 ± 2 02	6	(17) 206 ± 2.63	8	17 ± 3.3	5.2
Weight at 300 days, g.	(20) 205 ± 2.83	9	(17) 225 ± 3 90	11	20 ± 4.8	4.2
Males:						
Gain in weight, 28–56 days, g.	(20) 51 ± 1.9	26	(20) 80 ± 1.6	15	29 ± 2.0	14.5
Weight at 100 days, g.	(20) 186 ± 3.45	12	(20) 218 ± 3.77	11	32 ± 5.1	6.3
Weight at 210 days, g.	(20) 292 ± 3.80	9	(20) 309 ± 5.52	12	17 ± 6.7	2.5
Weight at 300 days, g.	(20) 308 ± 3.68	8	(20) 327 ± 5.28	11	19 ± 6.3	3.0

* Figures in parentheses indicate number of cases.
† Numbers vary due to exclusion of pregnant females, and to deaths.

diet, those showing more rapid growth in weight as an individual charac-
teristic tend to show the temporary low-calcium condition of body in a
more pronounced degree.[2]

Still further, it is found that when growth is accentuated by adding
protein—alone or as meat—to the already adequate basal diet, this tends
to induce a more marked low-calcium condition with symptoms suggestive
of those of calcium deficiency.[3] While in the majority of such cases there
is spontaneous recovery, some of the females which had been thus stimu-
lated to rapid growth and early puberty then broke down in reproduction.

The experimentation has therefore been continued so that our total
findings may be given a more quantitative character, the reporting of
which is the purpose of the present paper.

Of 40 young females (rats) on the basal diet (Laboratory Diet No. 16,
or Diet A), 3 or 7.5 per cent died between the ages of 1 month ("end of
infancy") and 1 year (early adulthood); while of 40 of the same age, sex,
heredity and nutritional background, but receiving a supplement of lean
meat in addition to the basal diet, 7 or 17.5 per cent died during the same
age period. The protein supplement in these cases was 5 (or in one series,
10) grams of lean meat on each of six days each week. This increased the
protein of the diet from about 14 per cent to about 24 per cent of the dry
matter of the food.

Quantitative Comparison of Biochemical Performance in the Life History. -
Findings on several points which have proved noteworthy criteria in the
judgment of biochemical well-being and nutritional performance are shown
quantitatively in tables 1 and 2.

The data summarized in table 1 show a marked increase of gain in body
weight when the basal diet of wheat and milk is supplemented with meat
as described above. The difference with females was 8.5, and with males
8.9, times its Probable Error. These Critical Ratios are very clearly
significant. This is strongly confirmed by the corresponding data in table
2 which includes the means of all available cases of experimental animals
on the protein-supplemented diet (Laboratory Diet 16 P 5) and of all the
most closely related—not always litter-mate—controls on Diet 16. Here
the increased growth due to the addition of meat to the basal wheat and
milk diet (Diet 16) was 11.2 times its Probable Error with females, and
14.5 times with males. Very clearly the findings of the two series of
experiments show that the difference in diet resulted in a difference in
growth which was undoubtedly statistically significant.

The protein supplement which thus increased the rate of growth appears
also to have stimulated an earlier development of puberty as shown by
earlier reproduction when the sexes grew up together. The observed
difference is 4.9 times its Probable Error in the series of 10 females with
10 litter-mate controls; and 3.6 times in the larger series with 28 experi-

mental females and 26 controls, all closely related though not all litter-mate controls.   Consistently with the usual interpretation in this field of research, we judge these differences to be statistically significant.

The numbers borne, and also the numbers reared, per female, were essentially the same on each of the two diets here compared.   The average weight of offspring at the age of 28 days (conventionally considered the end of infancy in the rat) was distinctly higher in the families receiving the supplementary meat ration.   The latter, however, seems to have had no clearly significant influence upon the duration of reproductive life as measured in the females or upon the lengths of life in the animals represented in table 1.   Some of the additional animals included in the data of table 2 are still living.

Consistently with their greater gains in early growth, the rats fed supplementary protein showed distinctly higher body weights at 100 days of age, but the differences were less pronounced at 210 and at 300 days.

Body weights at higher ages, average lengths of life of larger numbers, and the influence of combined supplementation of the diet with protein and calcium are being studied in experiments still in progress.

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DISTRIBUTION OF PRESSURE ON CURVED PROFILES IN SUPERSONIC GAS FLOW WITH VARIABLE ENTROPY

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1. Introduction.—A theory of pressure distribution on curved profiles immersed in a gas moving at supersonic speed was presented in a previous note in these PROCEEDINGS and pressures were calculated, to first approximations, for a circular arc profile based on this theory.[1]   The formal basis of the theory involves the representation of pressure as a function $p(\omega, S)$, where ω denotes the inclination of the stream lines and S the entropy of the gas, and the power series expansion of $p(\omega, S)$ about a point of inclination ω on the profile.   It was pointed out that the second approximation of pres-

sure required the integration of a more or less complicated differential equation of the first order and that this integration could best be performed by use of a high-speed computing machine which was not immediately available to us.

The discussion of the following note[3] is based on the possibility of expansion of the function $p(\omega, S)$ about points of the shock line assumed to originate at the vertex of the profile. Thus we consider the series

$$p(\omega, S) = p(\omega, S_*) + \frac{\partial p(\omega, S_*)}{\partial S} (S - S_*) + \frac{1}{2} \frac{\partial^2 p(\omega, S_*)}{\partial S^2} (S - S_*)^2 + \ldots$$

in which ω is the inclination of the stream line at a point immediately behind the shock line and S_* denotes the entropy at this point. Taking the arbitrary constant in the definition of the entropy function to be such that $S = 0$ for points on the profile, and denoting by $p = \theta(\omega)$ the pressure distribution over the profile, the above equation gives

$$\theta(\omega) = p(\omega, S_*) - \frac{\partial p(\omega, S_*)}{\partial S} S_* + \frac{1}{2} \frac{\partial^2 p(\omega, S_*)}{\partial S^2} S^2 - \ldots .$$

Termination of this series after its first term gives the relation $\theta(\omega) = p(\omega, S_*)$ used for the first approximation of pressure on the profile. Including the second term we have the relation

$$\theta(\omega) = p(\omega, S_*) - \frac{\partial p(\omega, S_*)}{\partial S} S_*. \tag{1}$$

Under the assumption that the flow is uniform in front of the shock line, the quantity S_* is an explicit function of ω alone. In §3 it will be shown that the derivative $\partial p(\omega, S_*)/\partial S$ is likewise given explicitly as a function of ω in view of the fact that the curvature K of the stream lines, which is naturally introduced by the process of differentiation, cancels from the several terms which make up the expression for this derivative. Hence it is feasible to determine the pressure along the profile from (1) by means of an ordinary computing machine.

2. *Résumé of Formulas for First Approximation of Pressure.*—By the first approximation formula the pressure at any point of the profile, at which the inclination is ω, is given by the function which expresses the pressure in terms of the inclination ω of the stream lines immediately behind the shock line. Denoting by p_1 the constant pressure of the uniform flow in front of the shock line we have

$$\frac{p(\omega, S_*)}{p_1} = \frac{2\gamma}{\gamma + 1} (M^2 \sin^2 \alpha - 1) + 1, \tag{2}$$

where M is the Mach Number of the uniform flow and $\gamma = 1.405$ for air;

also α is the inclination of the shock line and is related to the inclination ω of the stream lines by the formula

$$\tan \omega = \frac{2[(M^2 - 1) \tan^2 \alpha - 1]}{[(\gamma - 1)M^2 + 2] \tan^2 \alpha + [(\gamma + 1)M^2 + 2] \tan \alpha}. \quad (3)$$

Formulas (2) and (3) permit the determination of the first approximation of pressure. While the quantity S_* does not enter into this approximation, we note that it is given by $S_*(\omega) = Jc_v \log \Delta(\omega)$ where

$$\Delta(\omega) = \frac{(p/p_1)/(\rho/\rho_1)^\gamma}{(p_v/p_1)/(\rho_v/\rho_1)^\gamma}, \quad (4)$$

with

$$\left. \begin{aligned} \frac{p}{p_1} &= \frac{2\gamma}{\gamma + 1} (M^2 \sin^2 \alpha - 1) + 1, \\ \frac{\rho}{\rho_1} &= \frac{2(M^2 \sin^2 \alpha - 1)}{(\gamma - 1)M^2 \sin^2 \alpha + 2} + 1. \end{aligned} \right\} \quad (5)$$

Here J and c_v have the usual meaning, ρ_1 is the density in the uniform flow, while p and ρ are pressure and density at points immediately behind the shock line. Also p_v and ρ_v in (4) stand for pressure and density behind the shock line and at the vertex V of the profile; these quantities are therefore determined by (5) in which α is the inclination of the shock line at V.

3. *Derivation of the Formulas for the Derivatives of the Function $p(\omega, S)$ Along the Shock Line.*—To determine the derivative $\partial p/\partial S$ in (1) we first consider the relations[3]

$$\frac{\partial p}{\partial \omega} = \frac{dp}{d\sigma} \Big/ \frac{d\omega}{d\sigma} = \frac{dp/d\sigma}{K}, \quad (6)$$

$$\frac{dp}{d\sigma} = p_{,\alpha} \frac{dx^\alpha}{d\sigma} = -\rho A_\alpha \zeta_\alpha^i \frac{dx^\alpha}{d\sigma}, \quad (7)$$

at points immediately behind the shock line, where σ denotes arc length along the stream lines. But from the definition of the ζ_α^i it readily follows that $\zeta_\alpha^1 dx^\alpha/d\sigma = 0$ and $\zeta_\alpha^2 dx^\alpha/d\sigma = 1/v$, where v is the magnitude of the velocity. Hence from (7) we have

$$\frac{dp}{d\sigma} = -\rho A_{22} \zeta_\alpha^2 \frac{dx^\alpha}{d\sigma} = -\rho \frac{A_{22}}{v}, \text{ or}$$

$$\frac{dp}{d\sigma} = -\rho \frac{B_{22}K}{v} = \frac{-\rho B_{22}K}{vG_0(M, \alpha)}, \quad (8)$$

where we have put $A_{ij} = B_{ij}\kappa$, $K = G_0\kappa$ with κ the curvature of the shock line. But[3]

$$G_0 = -\frac{B_{12}}{vu_n} + \frac{B_{22}u_t}{v^2 u_n}$$

in which $B_{12} = 4u_n u_t/\gamma + 1$; here u_t denotes the tangential component and u_n the normal component of velocity behind the shock line. Hence

$$\frac{B_{22}}{vG_0} = vu_n\left[\frac{v}{u_t} + \frac{4}{(\gamma + 1)G_0}\right]$$

and making this substitution in (8) we have

$$\frac{dp}{d\sigma} = -\rho v u_n K\left[\frac{v}{u_t} + \frac{4}{(\gamma + 1)G_0}\right] \tag{9}$$

along the shock line.   Finally from (6) and (9) we find

$$\frac{\partial p}{\partial \omega} = -\rho v u_n\left[\frac{v}{u_t} + \frac{4}{(\gamma + 1)G_0}\right]. \tag{10}$$

It is to be observed from (10) that the derivative $\partial p/\partial \omega$ along the shock line is independent of the curvatures of the stream lines or shock line and in fact depends only on the inclination of the shock line for given uniform incident flow.

The formula for the derivative $\partial p/\partial S$ in (1) is now obtained by combining (10) with the following relation[4]

$$\left[(m^2 - 1)\left(\frac{\partial p}{\partial \omega}\right)^2 - \rho^2 v^4\right]\frac{d\omega}{d\sigma} = -\tau A \rho^2 v^2\frac{\partial p}{\partial S} \tag{11}$$

in which $\tau = (\gamma - 1)Jc_v$, m is the variable Mach Number for flow behind the shock line, and A is a certain quantity which is constant along stream lines.   At points immediately behind the shock line A is given by[5]

$$A = -\frac{[\rho]^2 u_t \kappa}{\rho_1 \rho p} = -\frac{[\rho]^2 u_t K}{\rho_1 \rho p G_0} \tag{12}$$

in which $[\rho] = \rho - \rho_1$ stands for the difference in the densities on the two sides of the shock line.   Making the substitutions $u_n = v \sin(\alpha - \omega)$, $u_t = -v \cos(\alpha - \omega)$ and putting

$$\beta = \frac{v}{u_t} + \frac{4}{(\gamma + 1)G_0} \tag{13}$$

in (10) it now follows from (10), (11) and (12) that

$$\frac{\partial p(\omega, S_*)}{\partial S} = \frac{[(m^2 - 1)\beta^2 \sin^2(\alpha - \omega) - 1]\rho v^2 G_0}{-\gamma\tau\left(\frac{\rho}{\rho_1} + \frac{\rho_1}{\rho} - 2\right) m^2 \cos(\alpha - \omega)} \tag{14}$$

Equation (14) gives the required formula for the derivative $\partial p/\partial S$ along the shock line. As mentioned before it is now seen that this derivative depends only on the inclination ω of the stream lines (or in consequence of (3) on the inclination α of the shock line). Using the relation

$$\frac{\rho v^2}{p_1} = \gamma M^2 \left(\frac{\rho}{\rho_1}\right) \frac{\cos^2 \alpha}{\cos^2(\alpha - \omega)},$$

which is easily derived, it follows that (1) can be given the form

$$\frac{\theta(\omega)}{p_1} = \frac{p}{p_1} + I \log \Delta \tag{15}$$

where,

$$I = \frac{M^2(\rho/\rho_1) \cos^2\alpha \, [(m^2 - 1)\beta^2 \sin^2(\alpha - \omega) - 1]G_0}{(\gamma - 1)\left[\frac{\rho}{\rho_1} + \frac{\rho_1}{\rho} - 2\right] m^2 \cos^2(\alpha - \omega)}.$$

The left member of (15) is the ratio of the pressure $\theta(\omega)$ along the profile to the pressure p_1 of the incident flow. The ratios p/p_1 and ρ/ρ_1 are given by (5). To evaluate $\log \Delta$ we have the equation (4) and G_0 can be evaluated from the explicit formula for this quantity.[8] Finally the Mach Number m is given by

$$m^2 = \frac{v^2}{c^2} = \frac{v^2}{u_n^2} \cdot \frac{u_n^2}{c^2} = \frac{u_n^2/c^2}{\sin^2(\alpha - \omega)}$$

in which the ratio u_n^2/c^2 has been calculated previously (see, "Calculation of the Curvatures of Attached Shock Waves," *loc. cit.*, ref. 3). Using these formulas in conjunction with the relation (3), it is possible to find, by means of (15), the pressure ratio $\theta(\omega)/p_1$ at any point of the curved profile.

4. *Calculations and Conclusions.*—The following table shows the values of the quantity $1000I \log \Delta$ at the indicated $\%$ chords and for the indicated angles of attack a in the case of the flow past the circular arc profile considered in our previous communication.[1] In carrying out these calculations α was chosen as the independent variable and computations were made for whole and intermediate angular values of this variable over the required ranges. It was found in general that the calculated numerical values of I increased with α but that deviations from this behavior occurred over a

restricted range corresponding to a range of values of ω about $\omega = 0$. Since it appeared that these irregularities were due to the limitation in the accuracy of the trigonometric tables used and since the deviations in question were small, it was decided to replace the calculated values of I in this α range by values obtained on the assumption that I could be considered to vary linearly with α over this range. The values of $1000I \log \Delta$ at the various % chords in the table were then obtained from calculated values of this quantity by interpolation.

% CHORD	$a = -10$	$a = 0$	$a = 28$
0.0	0.00	0.00	0.00
4.0	5.75	0.51	0.87
12.0	11.96	1.20	3.06
21.5	14.64	1.58	5.95
31.0	14.38	1.72	9.80
40.5	13.48	1.65	14.42
50.5	11.38	1.53	19.82
59.0	11.21	1.45	24.57
68.5	10.72	1 37	29.01
78.5	10.00	1 48	31.62
87.5	9.29	1.79	27.42
97.0	8.65	2.48	.....

It is seen that as we go down the columns for each value of a in the table the values of the quantity $I \log \Delta$ at first increase and then decrease. Actually in the case of the column marked $a = 0$ a certain oscillatory behavior in the values of this quantity occurs. However, the quantity $I \log \Delta$, representing the difference between the first approximation of pressure and the higher approximation under consideration, does not seem to have a direct physical significance, and hence it would not appear that this behavior is, in itself, of significance in the physical problem. Since the entries in the table give $1000I \log \Delta$ the values of $I \log \Delta$ are seen to be extremely small and are moreover small in comparison with the values of the first approximation of pressure except near the tail end of the profile for $a = 28$ where they are of the same order of magnitude as the first approximation. It may reasonably be expected that terms involving squares of entropy differences in the gas, which will occur in the next higher approximation, will be small in comparison with the quantity $I \log \Delta$; if this is correct it would appear that the values of pressure over the profile given by the first approximation are very near, except possibly for extreme values of the angle of attack, to the exact theoretical values under the assumption of zero viscosity and thermal conductivity.

[1] "First Approximation of Pressure Distribution on Curved Profiles at Supersonic Speeds," these PROCEEDINGS, **35**, 617–627 (1949).

[2] Prepared under Navy Contract N6onr-180, Task Order V, with Indiana University.

³ The formulas for the Γ'_a, the A_{ij} and the following quantity $G_6(M, \alpha)$ are given in the paper "On Curved Shock Waves," *J. Math. Physics*, 26, 62-68 (1947). A more explicit formula for G_6 is to be found in the article "Calculation of the Curvatures of Attached Shock Waves," *Ibid.*, 27, 279-297 (1949).

⁴ This relation is derived in §7 of the forthcoming article "The Determination of Pressure on Curved Bodies Behind Shocks," *Communications on Applied Mathematics*, *New York Univ., Inst. for Math. and Mech.*

⁵ See §5 of ref. 4.

THE SELECTIVE ADVANTAGE OF AN ADENINELESS DOUBLE MUTANT OVER ONE OF THE SINGLE MUTANTS INVOLVED*

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In discussions of evolutionary specialization it is often suggested that if one reaction in a biosynthesis is blocked, the ability to carry out other reactions preceding this one in the series will tend to be lost as a result of mutation, provided that the intermediates involved are not themselves useful. Such a tendency would be predicted if mutations which result in loss of synthetic ability were more frequent, or of greater selective advantage than those which restore the ability. The possibility that loss of synthetic ability may sometimes confer a selective advantage provides an interpretation of the behavior of the adenineless strain of Neurospora which is discussed here. In each of three stock cultures of this strain there occurred spontaneously a second mutation to adenineless, and the cultures became genetically pure for the double mutants. Selection of the double mutants may be accounted for by the fact that, on the medium used, the double mutant in each reaches its maximum rate of growth more quickly than does the original single mutant.

Detection of the Spontaneous Mutations.—A striking characteristic of the strain in which the new mutations appeared makes detection of the presence of certain other adenineless mutant genes quite simple. The accumulation by this strain (isolation number 35203) of a purple pigment is prevented if any one of three other adenineless mutant genes (isolation numbers 27663, 28610 and 44411) is present.[1] Available evidence indicates that the purple pigment is a derivative of an intermediate in the biosynthesis of adenine and that accumulation of the pigment is prevented by the 27663, 28610 and 44411 mutations because these interfere with reactions which come earlier in the series, thereby cutting off the supply of pigment precursor.[1] Hence

the single mutant, 35203, is purple while the double mutant of 35203 with 27663, 28610 or 44411 is not purple.

The three isolates of ad-p (purple-adenineless), which were observed to have lost the purple character but to have retained the requirement for adenine, had been kept as stock cultures at room temperature and had been transferred at six- to eight-week intervals. Some time after the disappearance of the purple character they were out-crossed to wild type and segregation of two adenineless mutants, one of which produced the purple pigment, was demonstrated in all perithecia tested. The double mutant in each case was not purple.

Comparison of Growth Rates of the Single and Double Mutants.— The genetic constitutions of the three double mutants obtained from the above crosses were checked by out-crossing them to wild type. Also double

TABLE 1

| | GROWTH RATES (MM./HR.) | |
STRAIN	COMPLETE MEDIUM	MINIMAL MEDIUM + ADENINE*
ad-p, isolate 1	2.9	3.5
ad-p, isolate 2	2.6	3 4
ad-p, isolate 3	2.8	3.4
ad-p, isolate 4	2.6	3.4
ad-p, isolate 5	2.8	3.5
ad-x1, ad-p	3.5	3.7
ad-x2, ad-p	3.5	3.6
ad-x3, ad-p	3.5	3.9
27663, ad-p	3.5	3.5
44411, ad-p	3.6	3 8
ad-p, isolate 1 + ad-x1, ad-p	3.5	3 0
ad-p, isolate 1 + ad-x2, ad-p	3.5	3.6
ad-p, isolate 1 + ad-x3, ad-p	3.5	3.8
ad-p, isolate 1 + 27663, ad-p	3.5	
ad-p, isolate 1 + 44411, ad-p	3.5	

* Adenine sulfate, 0.5 mg. per 10 ml. medium.

mutants of ad-p with two of the not-purple mutants, 27663 and 44411, were prepared. Growth rates were measured at 25°C. on complete medium in growth tubes.[3] Innocula were obtained from complete agar slant cultures which were from 7 to 10 days old. The tubes were marked at the mycelial frontier 15 to 20 hours after inoculation and the growth rate determined for the following 48-hour period. Results are given in table 1, where the three spontaneous mutations are designated as ad-x1, ad-x2 and ad-x3. It will be seen that on the complete medium the single mutant ad-p grows more slowly than any of the double mutants. Also, in tubes which were inoculated with a mixture of spores from ad-p and any one of the double mutants the rate of growth was the same as that of the double mutant. No purple pigment was formed in these tubes, except near the

point of inoculation. The growth rate of the purple strain is not constant during this 48-hour period, but is increasing, and after 60 to 70 hours approaches that of the other strains. However, it would appear that, in mixed cultures, the purple strain is forced out very early, since the pigment has been observed only very near the point of inoculation and the rate of growth is fairly constant 15 or 20 hours after inoculation.

The complete medium used had the same composition as that upon which the stock cultures had been kept. It consisted of the usual minimal medium[3] supplemented with an autolyzate of wild-type Neurospora mycelium, prepared as described by Lein, Mitchell and Houlahan.[4] A concentration of autolyzate equivalent to 40 mg. moist weight of mycelium per ml. of medium was used. On minimal medium supplemented with adenine there is little difference in the growth rate of ad-p and that of the other strains in the 48-hour period considered, but there is a greater difference during the first 15 hours after inoculation, the distance along the tube covered by ad-p being about two-thirds of that covered by the other strains. Apparently the difference is sufficient to allow selection of the double mutant on this medium too, since no pigment was observed in mixed cultures except near the innoculum.

In liquid culture, if the autolyzate is used as a supplement, after 30 to 40 hours growth the dry weight of mycelium from the double mutants and mixed cultures is 4 to 5 times greater than that from the purple mutant. After 50 to 60 hours the difference in dry weight is only about 16%, but no pigment was observed in the mixed cultures. When adenine was used as a supplement (1 mg. adenine sulfate per 20 ml. of medium) there was little difference in the dry weights after 40 hours, and the mixed cultures were purple.

Genetic Relationships of the Spontaneous Mutants and the Induced, Not-Purple Adenineless Mutants.—From crosses of the double mutants to wild type the three new mutants, ad-x1, -x2 and -x3 were obtained without ad-p. They were crossed with each other, with 27663, 28610 and 44411, and with a fourth not-purple adenineless mutant, 3254, which gives a faintly purple double mutant with ad-p. The crosses were made on corn meal agar slants and the ascospores obtained were suspended in sterile water and spread on agar minimal medium in petri-plates. The plates were kept at 25°C. for 12 to 14 hours after heat treatment and then examined for wild-type spores. These can be distinguished from mutant spores since the latter produce very short haphae and then stop growing on the unsupplemented medium, while growth of the wild type continues.[4]

From the results of these crosses, given in table 2, it appears that the three spontaneous mutants are all genetically different and that ad-x2 is also different from the four induced not-purple mutants. It is possible that ad-x1 is allelic with 28610 and ad-x3 with 27663; fertile crosses were not obtained in these two cases.

Discussion.—It is clear that in mixed cultures, containing ad-p and a double mutant of ad-p with one of the not-purple adenineless mutants described, the double mutant is selected on either of the two agar media used. The slower initial growth rate characteristic of ad-p appears to be adequate reason for the selection, but is possible that other less obvious factors are involved. The cause of the reduced growth rate is not known. Possibly the accumulation of pigment is in some way responsible but this has not been demonstrated. It can only be said that a mutation which prevents the accumulation of pigment removes, or compensates for, a detrimental effect of the ad-p mutation.

Fries, working with Ophiostoma, has observed three cases in which a mutant strain, after having spontaneously acquired a second mutation, has become genetically pure for the double mutant. Two of these strains were guanine-requiring and the second mutation in both was to hypozanthineless.[5] The third was an adenineless mutant in which a mutation to biotinless occurred.[6] These, of course, differ from the case reported·here in that new growth requirements are introduced, but knowledge of possible interrelations in the biosyntheses concerned might diminish this difference.

TABLE 2

STRAINS	WILD-TYPE PROGENY OBSERVED	STRAINS	WILD-TYPE PROGENY OBSERVED
ad-x1 × ad-x2	++	ad-x2 × 27663	++
ad-x1 × ad-x3	++	ad-x2 × 28610	++
ad-x1 × 3254	++	ad-x2 × 44411	++
ad-x1 × 27663	++	ad-x3 × 3254	++
ad-x1 × 28610	No fertile cross	ad-x3 × 27663	No fertile cross
ad-x1 × 44411	++	ad-x3 × 28610	++
ad-x2 × ad-x3	++	ad-x3 × 44411	++
ad-x2 × 3254	++		

One may question the usefulness of speculation upon the applicability of selection of a further loss of synthetic ability as a mechanism for evolutionary specialization. The observations of Fries and those described here serve to establish that under certain laboratory conditions the phenomenon of selection does take place. Possibly it does so to a significant extent under natural circumstances as well.

It is of interest to compare the mutations which prevent the accumulation of purple pigment by ad-p with suppressors which operate on Drosophila· mutants characterized by abnormally high pigment production. If the adenineless strains of Neurospora were always kept on a complex medium, and the adenine requirement not therefore recognized, then the not-purple mutations which prevent pigment production would, if they were encountered, be looked upon as suppressors of the purple character, quite analogous to suppressors of black,[7] sable[8] and perhaps others, in Drosophila. There

might be found then, at least four complete suppressors and one partial suppressor of purple, namely 27663, 28610, 44411, ad-x2 and 3254. Furthermore, there is another genetically distinct adenineless mutant, 44206, previously described,[1] but not otherwise discussed, here, which produces the purple pigment under different conditions and to a lesser extent. Hence this strain is quite different in appearance from ad-p and it might therefore be classified as an unrelated mutant suppressed by the same series of suppressors which operate on ad-p.

Summary.—In three stock cultures of an adenineless strain of Neurospora a double mutant arose as the result of spontaneous mutation to adenineless, and, in each case, the double mutant forced out the original single mutant. Under conditions which existed at the time the selection took place the double mutants show a faster initial rate of growth than the original single mutant. The three new mutations are genetically distinct and each one, in combination with the original mutant prevents accumulation of a purple pigment which is characteristic of this strain. Cultures from mixed innocula of the purple mutant with any one of the double mutants have the growth rate of the double mutant and form no pigment.

[1] Mitchell, H. K., and Houlahan, M. B., *Fed. Proc.*, **3**, 370 (1946)
[2] Ryan, F. J., Beadle, G. W., and Tatum, E. L., *Am. J. Bot.*, **30**, 784 (1943).
[3] Beadle, G. W., and Tatum, E. L., *Ibid.*, **32**, 678 (1945).
[4] Lein, J., Mitchell, H. K., und Houlahan, M. B., Proc. Natl. Acad. Sci., **34**, 435 (1948).
[5] Fries, Nils, *Physiologia Plantarum*, **2**, 78 (1949).
[6] Fries, Nils, *Proc. 8th Intern. Congr. Genetics*, Stockholm (in press), 1949.
[7] Plough, H. H., *Verh. d. V. Intern. Kongr. f. Vererbung, Berlin I.*, **1**, 193 (1928).
[8] Schultz, J., and Bridges, C. B., *Am. Nat.*, **66**, 323 (1932).

HIGHER PROPERTIES OF PHYSICAL SYSTEMS OF CURVES

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1. A *system* S_k of ∞^3 curves in a field of force consists of curves along which a constrained motion is possible such that the pressure P is proportional to the normal component N of the force vector. Thus $P = kN$ where $k(\neq -1)$ is the constant factor of proportionality.[1]

A system S_k, where $k \neq -1$ but may be infinite, of ∞^3 curves is defined by the ordinary differential equation of third order

$$(\psi - y'\phi)y''' = [\psi_x + (\psi_y - \phi_x)y' - \phi_y y'^2]y''$$
$$- \left[3\phi - \frac{2k(\phi + y'\psi)}{(1 + k)(1 + y'^2)}\right]y''^2, \quad (1)$$

where $\phi(x, y)$ and $\psi(x, y)$ are the horizontal and vertical components of the force vector.

The important special cases of physical interest are (a) dynamical trajectories, $k = 0$; (b) general catenaries, $k = 1$; (c) generalized brachistochrones, $k = -2$; (d) velocity curves, $k = \infty$.

2. At any lineal element (x, y, y'), the speed of a particle traversing those curves of a system S_k that have four-point contact with their circle of curvature is called the *hyperosculating speed*.

The hyperosculating speed v at any lineal element (x, y, y') is determined by the formula

$$v^2 = \frac{(3 + k)(\psi - y'\phi)(\phi + \psi y')}{\psi_x + (\psi_y - \phi_x)y' - \phi_y y'^2}. \quad (2)$$

The corresponding hyperosculating radius of curvature is

$$r = \frac{(3 + k)(\phi + \psi y')(1 + y'^2)^{1/2}}{(1 + k)[\psi_x + (\psi_y - \phi_x)y' - \phi_y y'^2]}. \quad (3)$$

3. Consider a lineal element E through a given point P, which is not perpendicular to the direction of the line of force through P. In a system S_k where $k \neq -1, -3$, there is a *single* curve, actual or virtual, which passes through E such that it has four-point contact with its circle of curvature at E.

PROPERTY III. *The locus of the centers of the ∞^1 hyperosculating circles constructed at a given point P for all elements is a conic passing through P in the direction of the force vector.*

The equation of this conic in running coordinates (X, Y) is

$$(1 + k)[\phi_y(X - x)^2 + (\psi_y - \phi_x)(X - x)(Y - y) - \psi_x(Y - y)^2] +$$
$$(3 + k)[-\psi(X - x) + \phi(Y - y)] = 0. \quad (4)$$

PROPERTY IV. *The normal to the conic (4) of Property III at the point P cuts the conic again at a distance equal to $(3 + k)/(1 + k)$ times the radius of curvature of the line of force which passes through P.*

These results were originally stated by Kasner in his Princeton Colloquium Lectures. In the present paper, we shall discuss some new results which are related to the Properties III and IV.

4. At any point P, the *exceptional directions* of a given field of force are given by the quadratic equation in y',

$$\psi_x + (\psi_y - \phi_x)y' - \phi_y y'^2 = 0. \tag{5}$$

A point P is *exceptional* if every direction through P is exceptional. At a non-exceptional point P, there are two exceptional directions, real or imaginary.

In a field of force, which is neither constant nor elastic, there is a net of $2 \infty^1$ exceptional curves. This exceptional net is orthogonal if and only if the field of force is conservative.

The direction of the line of force at a point P is exceptional if and only if its curvature is zero at P. The direction orthogonal to that of the line of force at P is exceptional if and only if the magnitude of the force vector at P is instantaneously stationary upon moving P in this orthogonal direction.

For a field of force, the exceptional net consists of its lines of force and their orthogonal trajectories if and only if the lines of force are straight and the magnitude of the force vector is constant along each of the orthogonal trajectories.

All central fields of force belong to the above class.

The hyperosculating speed v for any system S_k where $k \neq -1, -3, \infty$, is a finite non-zero constant if and only if the lines of force are straight lines orthogonal to a fixed proper, or null, or rectilinear circle Γ such that the magnitude of the force vector at any point P is inversely proportional to the distance PQ where Q is the inverse or anti-inverse point of P with respect to Γ.

5. A point P is *ordinary* if it is non-exceptional and if the hyperosculating speed v at P depends not only on P but also on the direction y' through P.

Through a non-exceptional point P, consider a direction which is neither exceptional nor the direction of the line of force nor its orthogonal direction. If v_1 and v_2 denote the hyperosculating speeds in this direction corresponding to the two systems S_{k1} and S_{k2}, respectively, then

$$\frac{v_1^2}{v_2^2} = \frac{3 + k_1}{3 + k_2}. \tag{6}$$

6. As the direction y' varies about an ordinary point P, the corresponding hyperosculating speed v assumes various values. The directions, along which this speed v is instantaneously stationary, satisfy the quadratic equation in y',

$$[\psi_x(\psi^2 - \phi^2) - \phi\psi(\psi_y - \phi_x)] + 2\phi\psi(\phi_y - \psi_x)y' + [\phi_y(\psi^2 - \phi^2) - \phi\psi(\psi_y - \phi_x)]y'^2 = 0. \tag{7}$$

These are called the *principal directions.* At a non-ordinary point P, every direction is a principal direction. At an ordinary point P, there are only two principal directions, which may be real or imaginary.

For a field of force, which is not of the types described in Section 4, there is a principal net of $2 \infty^1$ curves.

At an ordinary point P, the hyperosculating speed v is instantaneously stationary along each of the two principal directions (7), which are symmetrically situated with respect to the line of force.

The following formula is analogous to Euler's formula for the normal curvatures of a surface.

At an ordinary point P, let λ_j, for $j = 1, 2$, denote the angle between any direction y' and a principal direction y_j, and let v and v_j denote the hyperosculating speeds corresponding to these directions y' and y_j'. Then

$$\frac{v^2 - v_1{}^2}{v_1{}^2 \sin^2 \lambda_1} = \frac{v^2 - v_2{}^2}{v_2{}^2 \sin^2 \lambda_2}. \tag{8}$$

Our final result is related to the Property IV stated above.

At a direction of a non-exceptional point P, which is neither exceptional nor the direction orthogonal to that of the line of force, the ratio ρ of the hyperosculating curvature κ of a system S_k to that of the hyperosculating curvature κ_0 of the velocity system S_∞ is

$$\rho = \frac{\kappa}{\kappa_0} = \frac{1 + k}{3 + k}. \tag{9}$$

Let the direction in the above result be taken as that of the line of force. Then κ_0 is the curvature of the line of force and κ is the curvature of the rest trajectory of the system S_k. Thus this result reduces to the theorem of Kasner concerning the comparative curvatures of the rest trajectory of a system S_k and the line of force.[2]

[1] Kasner, "Differential Geometric Aspects of Dynamics," Princeton Colloquium Lectures, Published by *Am. Math. Soc.*, 1913, 1934, 1948. See also a series of papers by Kasner and De Cicco published in these PROCEEDINGS, 1947–1949. The five characteristic properties of trajectories and general physical systems are there discussed. Property I relates to the circular focal locus, Property II to the angular relation, Property V to the variation of the conic of Property III, Properties III and IV are stated in the present paper.   Projective characteristics have been given by Terracini for trajectories.

[2] Kasner and Mittleman, "A General Theorem on the Initial Curvatures of Dynamical Trajectories," these PROCEEDINGS, **28**, 48–52 (1942). Kasner and De Cicco, "Physical Curves in Generalized Fields of Force, *Ibid.*, **33**, 160–172 (1948). De Cicco, "Constrained Motion upon a Surface under a Generalized Field of Force," *Bull. Am. Math. Soc.*, **53**, 993–1001 (1947).

THEORETICAL RELATIONSHIPS AMONG SOME MEASURES OF CONDITIONING

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The relationships among the various measures of strength of conditioning constitute an important problem for conditioning theory. Many different measures have been used.[1] The measures *latency* and *magnitude* are based on the occurrence of a single response, while *number of responses in extinction* and *the rate of responding* in a "free-response" situation are based on more than one instance of a response. *Probability of response occurrence* is another term that is encountered in the literature; it is used most frequently in cases where more than one response is possible (e.g., right and left turns in a T maze) and in circumstances when it is possible to compute the frequency or the percentage of times that a specified response is given. Percentage of response occurrence is taken to be an estimate of the probability of obtaining the response.

Some theoretical formulations are concerned with one or two measures of strength; others are more inclusive. In only few cases has an attempt been made to present a theory of the relation among measures. In most treatments that consider several measures, the relations among the measures are empirically determined.

The purpose of the present note is to indicate one possible theoretical account of the relationships among latency of response, rate of responding and the probability of occurrence of a response. The last measure serves as the starting point for the discussion and provides the terms in which the other concepts are related.

Consider the Skinner bar-pressing situation[2] in which a rat's responses may occur at any time and at any rate during the period in which the animal is in the experimental cage. Assume that the responses under constant testing conditions are randomly distributed in time. Let the rate of occurrence of these responses be represented by r. It may then be shown that the probability, $P_{>t}$, of obtaining an interval between two responses greater than t is

$$P_{>t} = e^{-rt} \qquad (1)$$

where e is the base of Naperian logarithms.[3] The probability of obtaining n responses in an interval, T, is

$$P_n = (rT)^n e^{-rT}/n! \qquad (2)$$

Equation (1) gives us a statement of the distribution of time intervals associated with various rates of responding. For example, for the median

time interval $P_{>t}$ is 0.5 and $-rt$ is $\log_e 0.5$ or the median t is $0.69/r$. Equation (2) gives the probability of various numbers of responses within some specified time interval. For example, the probability of getting exactly one response in an interval, T, is $(rT)e^{-rT}$. The relation between equations (1) and (2) is obvious when we consider the probability of getting no responses in an interval, T. In this case P_0 is e^{-rT}.

Equations (1) and (2) permit us to transform a rate measure into a probability measure. Since we are dealing with a continuous distribution (time), the probability of a response at any particular time is zero, but the probability of a response within given time intervals is finite and specifiable.

Latency usually refers to the time interval between a stimulus and a response and thus is not directly considered in the previous development. Assume, however, that the stimulus conditions are one determinant of the rate of responding, that is, that the rate has different values for different stimulus conditions. This assumption is consistent with the discussions by Skinner and others who have emphasized the measurement of rate; the assumption would presumably be an elementary requirement for any measure.

Under the circumstances of the assumption, t may be employed in discussing latency, since the latter would be the time interval between the beginning of the observation period (when a stimulus was presented) and the first response. Thus, on the assumption that stimulus conditions are a determinant of rate of responding and on the previous assumption that the responses are randomly distributed in time, a statement of the rate of responding under specified stimulus conditions implies a probability statement of the delay of length t between the presentation of the stimulus and the occurrence of the first response. This statement tells us not only of the distribution of latencies but also of the relationship between some representative value, say the median latency, and the rate of responding; for example, the probability of a response greater than the median latency, t_{md}, is 0.5; and from equation (1) we see that $-rt_{md} = \log_e 0.5$ or that the median latency equals $0.69/r$.

The preceding development does not imply any particular theory of conditioning but may be incorporated into a large class of theories. For example, if the foregoing discussion is combined with a theory that states that rate of responding is proportional to the number of responses that remain to be given in extinction, the measure of number of responses in extinction is immediately related to our latency and probability terms. In other words, if

$$r = k(N-n), \qquad (3)$$

where N is the number of responses in extinction, n is the number of re-

sponses already given, and k is a constant, we may substitute $k(N-n)$ for r in equation (1) and obtain

$$P_{>t} = e^{-k(N-n)t}. \tag{4}$$

This equation may then be examined for relationships existing among the terms n, N, P and t. In addition to the relationships among latency, rate and number of responses in extinction, equation (4) may be used to predict the distribution of responses in extinction for a constant strength and the distribution of time intervals between responses at various stages of extinction.

Since the present argument follows mainly from the assumption of a random distribution of responses in time, it is of interest to examine data
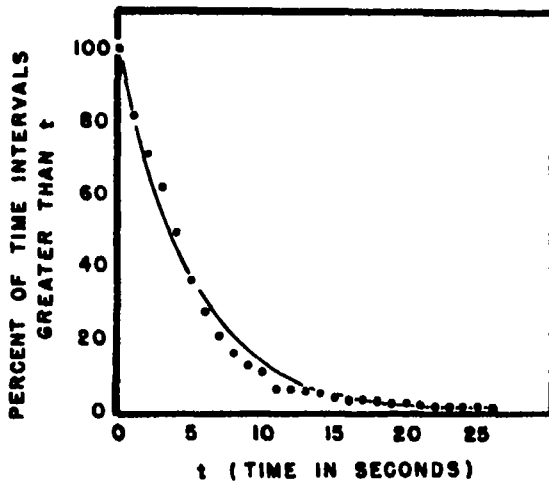
FIGURE 1

The percentage of inter-response time intervals greater than t, where t is time in seconds. The data are from an experiment with white rats in a bar-press situation as described in the text. The line drawn through the data is a plot of equation (1).

for direct evidence of randomness as well as for evidence relating to the above outlined consequences of randomness.

The data in figure 1 were taken from measurements obtained during the course of periodic reconditioning.[4] The data represent the responses of a single animal during a 20-minute session of "three-minute" periodic reconditioning. Within this observation period the rate of responding was approximately constant. The question at issue is whether the responses in this interval are distributed randomly. Equation (1) states that the probability of getting an interval between responses greater than t is e^{-rt}, where r is the rate of responding expressed in the same units as t. In the

20-minute session, 238 responses were made, 237 time intervals were recorded, and the rate in this session is 0.20 response per second. Thus, without direct reference to the distribution of time intervals, theory specifies the distribution of time intervals between responses uniquely. In this case the probability of getting a time interval greater than t (in seconds) is $e^{-0.20t}$. The ordinate of figure 1 shows the percentage of the intervals between responses that were greater than the various time values specified on the abscissa. The solid line through the date in figure 1 represents the theoretical function. The data are consistent with the assumption that the measured responses occurred randomly in time.

Although the data of figure 1 may be representative of the agreement between data and theory under the conditions specified, certain cases of systematic deviations from theory may be noted. One class of deviations, for example, may be found in cases where animals show marked "holding" behavior, i.e., where the bar is depressed and held down for many seconds. Although the "holding" period is not a "refractory" period[1] in the usual sense of the term, it obviously affects the data in a similar way. During the "holding" period, the probability of response occurrence is zero. One complicating feature in analyzing responses characterized by "holding" is the fact that "holding" is of variable length. The data available at present do not warrant an extensive treatment of this problem, but the simplicity that may result from apparatus changes designed to eliminate the factor of "holding" and the advantages that may accrue from the additional response specification may be shown.

An example of a distribution showing systematic deviations from theory is shown in figure 2. The computations and plot are similar to those in figure 1. The ordinate represents the percentage of intervals between responses greater than the specified abscissa values. The solid line is theoretical. The constant of the line was determined, as in the case in figure 1, directly from the rate of responding without reference to the distribution of time intervals. The fit is obviously poor; the function appears sigmoid and asymmetric.

Let us assume that the analysis leading to equation (1) and applied to figure 1 is correct when applied to all portions of the observation period except the time spent in "holding." An additional test may then be applied to the data from which figure 2 was obtained. Now we are interested in the measurement of the time interval between the end of one response and the beginning of the next.[2] Figure 3 shows the results of such measurements in the form of a plot of the percentage of intervals between the end of one response and the beginning of the next that were greater than the specified abscissa values. The solid line through the data is theoretical when the rate term, r, is set equal to the ratio of the number of responses to the total time minus the "holding" time, i.e., to the number of responses
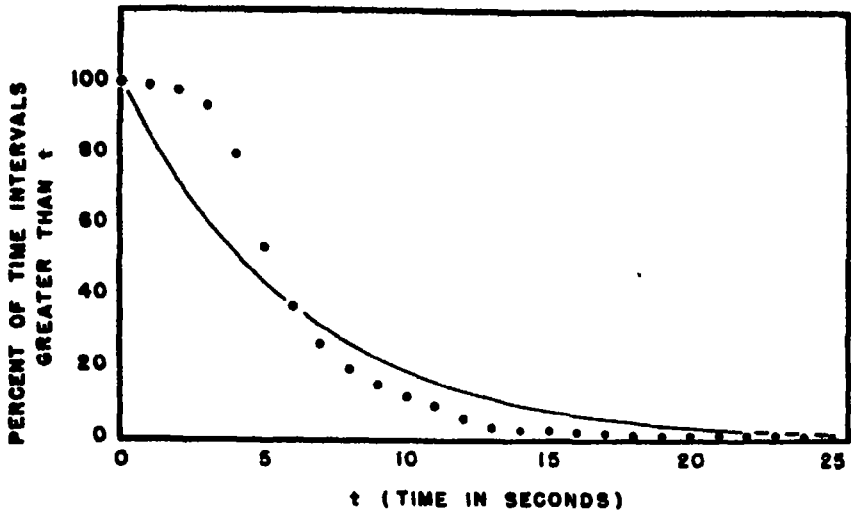
FIGURE 2

A plot similar to figure 1 showing the deviation from theory in cases of "holding" be-havior. The line drawn through the data is a plot of equation (1).
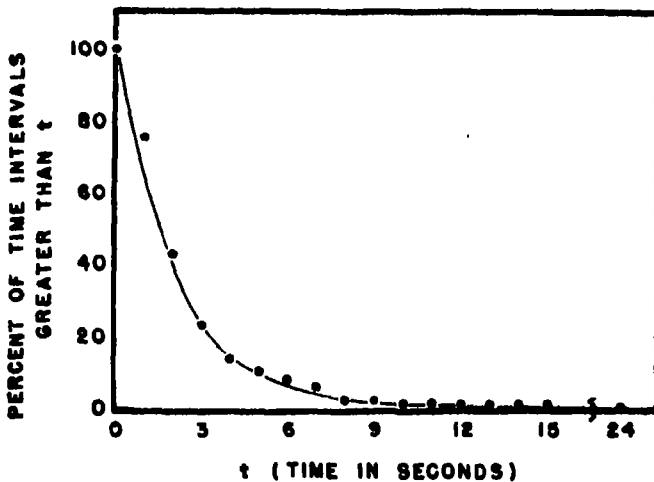
FIGURE 3

The data of figure 2 "corrected for holding." The plot is similar to that in figures 1 and 2, except that the measured interval is the time between the end of one response and the beginning of the next response. The line drawn through the data is a theoretical one described in the text.

per unit of "available" time.   As in figures 1 and 2 the constant is evaluated independently of the shape of the distribution of intervals.

Data relevant to the present analysis of latency measures are not numerous.   The agreement between the present theory and the data reported by Felsinger, Gladstone, Yamaguchi and Hull[6] is shown in figure 4, where the percentage of latencies greater than specified abscissa values are plotted. The solid line is the theoretical curve.   In the case of the latency data under consideration it is not possible to evaluate r independently of the distribution of time intervals.   In the case of figure 4 the constant was determined by the slope of a straight line fitted to a plot of $\log_e P_{>t}$ against t.
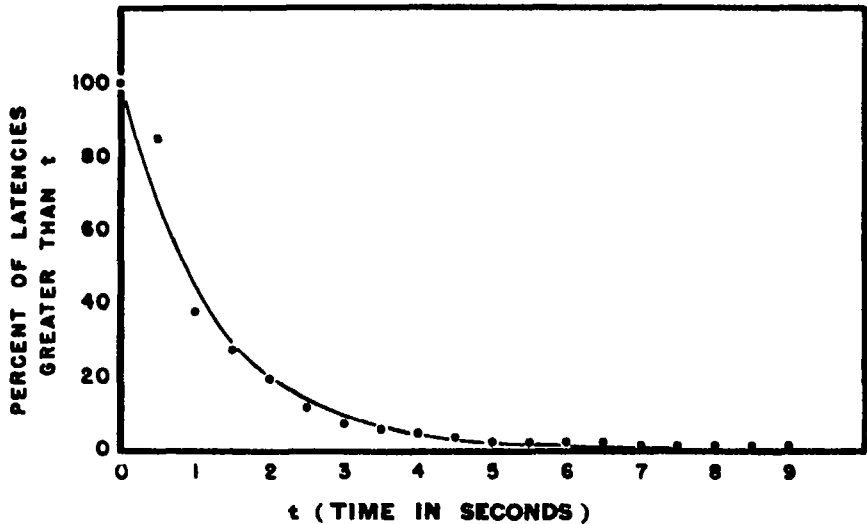
FIGURE 4

The percentage of latencies greater than t.   The data are from figure 1 of Felsinger, Gladstone, Yamaguchi and Hull.[6]

Probably little is to be gained at this time by further sampling of the consequences of equation (1), but many additional tests of the formulation may be made.   For some tests appropriate data are not available.   For the tests that have been tried the agreement between data and theory is promising.   One prediction that has been tested concerns the distribution of time intervals between responses for a number of animals at comparable stages in extinction.   The expectation is that at a specified stage in extinction the intervals between, say, response R_n and R_{n+1}, for a large number of animals, will be distributed in a manner similar to that shown in figure 1 and that the constant, r (therefore the steepness of drop of the curve) will vary systematically with n.   In other words, the steepness of the drop of a curve such as found in figure 1 will depend on where in extinction the inter-

vals are measured.   In fact this expectation seems to be borne out by the cases measured, although the number of measurements at each stage of extinction is not large.

Finally, it may be pointed out that the form of the present account has important consequences for the treatment of experimental data.   Since one of the features of the account is the possibility of specifying the frequency distributions of the measures discussed it is possible to eliminate many of the problems associated with the arbitrary selection of representative values in summarizing data.   On the basis of the preceding equations, one may state changes in one statistic, say the arithmetic mean, in terms of changes in another, say the geometric mean or the median.   Therefore, data using different statistics are made comparable and the multiplicity of functions that may arise from the use of different descriptive statistics not only ceases to pose a difficult problem but is actually an aid to theory testing.

Summary.—A theoretical account of some relationships among measures of strength of conditioning has been considered.   (1) If we assume that responses in a "free-response" situation are randomly distributed in time, we obtain directly a statement of the probability of occurrence of a response (or of any number of responses) within a specified time interval as a function of the length of the interval and of the rate of responding; we also obtain a statement of the probability of occurrence of inter-response time intervals of varying lengths.   (2) If we assume that, for any specified stimulus condition, there corresponds some rate of responding, it turns out that the probability of occurrence of latencies of various lengths may be specified for various rates of responding, or, for a fixed probability value, the relation between latency and rate may be specified.   (3) Finally, where these considerations are added to a theory specifying the relationship between rate of responding and number of responses yet to occur, the number of responses in extinction may be related to the latency and probability terms as well as to rate.   In addition to statements about average values, the present formulation has consequences for the distribution of time intervals between responses and, by extension, for the distribution of latency measures.

[1] Hull, C. L., *Principles of Behavior*, D. Appleton-Century Co., New York, 1943.

[2] Skinner, B. F., *The Behavior of Organisms*, D. Appleton-Century Co., New York, 1938.

[3] A slightly different equation results if we assume that a "refractory" period exists, i.e., that immediately after a response there is a period during which the probability of getting a response is zero. If we assume that the transition from the "refractory" period to randomness is instantaneous, the probability of getting an interval greater than *t* is

where t_0 is the "refractory" period. The formulation is more complex if the transition is treated as a gradual one or if the "refractory" period has a variable length.

⁴ The data reported here were recorded by Mr. Michael Kaplan in the Psychological Laboratories of Columbia University.

⁵ This is merely a first approximation. Subsequent analyses may show that the interval between the end of one response and the beginning of the next is not independent of the "holding" period. The results of our procedure indicate that the approximation is useful for the present.

⁶ The experiment by Felsinger, Gladstone, Yamaguchi and Hull [*J. Exptl. Psychol.,* 37, 214–228 (1947)] may not provide an optimal test of our formulation for two reasons. The first is that the data are reported in a frequency distribution with step intervals which begin at zero. If the shortest latency were greater than zero, starting the step intervals at the lowest measure would be more appropriate. The use of zero as a lower limit could easily make an exponential distribution more normal. The method of summarizing the data may account for the deviation of the point at 0.5 second in figure 4. The deviation of this point is an expression of the fact that the distribution reported by Felsinger, Gladstone, Yamaguchi and Hull does not have a maximum frequency at the first step interval.

In the second place, it may be assumed that the many transient discriminative stimuli associated with the exposure of the bar may play a more important rôle than the continuous ones associated with the presence of the bar. Although it is possible to extend the present notion to stimuli of short duration which end before the occurrence of the response, additional assumptions are required. A less equivocal test of the present theory may be expected from a distribution of latencies obtained from an experimental procedure of the sort used by Skinner (op. cit.), Frick [*J. Psychol.,* 26, 96–123 (1948)] and others. After a period of, say, no light, a light is presented and stays on until one response occurs (Skinner) or stays on for some fixed period of time sufficiently long to insure the occurrence of many responses (Frick). Such experimental procedures would minimize unspecified transient stimuli and would parallel more closely the notion that stimulus conditions determine a rate of responding. The procedure used by Frick has the additional advantage of permitting the measurement of the time interval between the onset of the stimulus and the first response and the subsequent intervals between responses under "the same" stimulus conditions.

PARTIAL DIFFERENTIAL EQUATIONS AND GENERALIZED ANALYTIC FUNCTIONS

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1. *Introduction.*—In this note (an abstract of results to be published in full elsewhere) we present the fundamentals of a theory of complex-valued functions $f = u(x, y) + iv(x, y)$ whose real and imaginary parts are connected by the equations

$$u_x = \sigma(x, y)v_y, \; u_y = -\sigma(x, y)v_x, \tag{1}$$

a theory which parallels closely that of analytic functions.

As early as 1891 Picard[1] foresaw the possibility of such a theory, but his suggestion seems to have remained unnoticed. Several years ago Gelbart and the author[2] considered the case $\sigma = a(x)b(y)$. In this case there exists an integration process (used implicitly by Beltrami[3] in a special case) which yields explicit formulas for solutions of (1) corresponding to the powers of a complex variable. From these all other single-valued regular solutions can be constructed, at least locally, by means of "formal power series." Our methods could be extended to systems of higher order (Diaz[4]) and to equations in three-space (Protter[5]), but they seem to be inadequate for the treatment of singular and multiple-valued solutions. The general case (1) has been considered recently by Bergman[6] and by Markushevitch (as reported by Petrovskii[7]), and especially important results have been obtained by Položii.[8] In the present paper we do not construct solutions in closed form as was done in the special case mentioned above, but are able to obtain considerably stronger general results. We shall impose only very mild regularity conditions on the coefficient $\sigma(x, y)$, but in order to achieve as close an analogy as possible with classical function theory we shall require at present that these conditions be satisfied in the whole function-theoretical plane. Most of our results, however, imply theorems of a local character.

It is hardly necessary to point out that the analogy between analytic functions and solutions of partial differential equations of elliptic type has inspired many recent investigations, notably the work of Bergman and Schiffer[9] on the kernel-function, and the theory of mappings by means of solutions of systems of differential equations developed by Lavrentyeff, Schapiro, and Gergen and Dressel.[10]

2. *Pseudo-Analytic Function.*—We set $x + iy = z$ and write functions of x and y as functions of z, without implying by this an analytic dependence on z. A function $\sigma(x, y) = \sigma(z)$ defined in a bounded domain D is called admissible if $\sigma > 0$, and σ_x and σ_y exist and satisfy a Hölder condition. A function σ defined over a Riemann surface F is admissible, if in the neighborhood of each point of F it is admissible as a function of the local parameter. In the following we use a fixed function σ defined and admissible over the whole complex plane (Riemann sphere). Thus $\sigma(z)$ is admissible for $|z| < +\infty$ and $\rho(\zeta) = \sigma(1/\zeta)$ is admissible for $|\zeta| < +\infty$.

We say that a complex-valued function $f(z) = u(x, y) + iv(x, y)$ is pseudo-analytic with respect to σ at a point z_0 if in some neighborhood of this point the partial derivatives of u and v exist, are continuous and satisfy (1). A function is pseudo-analytic in a domain if it is pseudo-analytic at every point in the domain. Complex constants are pseudo-analytic, so are linear combinations of pseudo-analytic functions with real coefficients.

Since equations (1) preserve their form under a conformal transformation, pseudo-analyticity can be defined over an arbitrary Riemann surface.

We shall make almost no use of this fact at present, except for the case of the Riemann sphere.

With each function $g(z) = \varphi(x, y) + i\psi(x, y)$ we associate the "reduced" function $^{\sigma(z)}g(z) = \sigma(x, y)^{-1/2}\varphi(x, y) + i\sigma(x, y)^{1/2}\psi(x, y)$. Using this notation we may express pseudo-analyticity by a differentiability requirement.

THEOREM. *A function $f(z)$ defined in a bounded domain D is pseudo-analytic in D if and only if for every z_0 in D the function $^{\sigma(z)}[f(z) - f(z_0)]$ (considered as a function of the complex variable z) is differentiable at z_0.*

The proof of this theorem requires much of the theory developed below.

3. Behavior at a Point.—The following theorem contains and sharpens some of the recent results by Položii. Our proof uses a method due to Carleman.[11]

THEOREM. *Let $f(z)$ be single-valued, pseudo-analytic and not a constant for $0 < |z - z_0| < r$. Then either $f(z)$ is pseudo-analytic at z_0 and $^{\sigma(z)}[f(z) - f(z_0)] \sim a(z - z_0)^n$, where $a \neq 0$ and n is a positive integer, or for some positive integer n, $^{\sigma(z)}f(z) \sim a(z - z_0)^{-n}$, $a \neq 0$, or $f(z)$ comes arbitrarily close to any given value in every neighborhood of z_0.*

In the first case we say that $f(z)$ assumes the value $f(z_0)$ with multiplicity n, in the second that $f(z)$ has at z_0 a pole of order n. In the third case (essential singularity) Picard's theorem holds by virtue of a result by Grötzsch,[12] since mappings by pseudo-analytic functions are mappings of bounded eccentricity.

COROLLARY. *Pseudo-analytic functions are interior transformations in the sense of Stoïloff.*

The behavior of a pseudo-analytic function at $z = \infty$ may be described in a similar manner.

4. Formal Powers.—Let r be a real rational number, $r \neq 0$, $|r| = p/q$, where p and q are relatively prime positive integers. Let a and ζ be complex numbers, $a \neq 0$. We say that a function $f(z)$ is a formal power with exponent r, coefficient a and center at ζ, and write $f(z) = Z^{(r)}(a, \zeta; z)$, if $f(z)$ is q-valued and pseudo-analytic for $0 < |\zeta - z| < +\infty$, if $w = f(z)$ is a one-to-one mapping of the q-times covered z-plane (with branch-points at ζ and ∞) onto the p-times covered w-plane (with branch-points at 0 and ∞) such that $f(\zeta) = 0, f(\infty) = \infty$ if $r > 0, f(\zeta) = \infty, f(\infty) = 0$ if $r < 0$, and if for $z \to \zeta$ we have that $^{\sigma(z)}f(z) \sim a(z - \zeta)^r$. We also set $Z^{(0)}(a, \zeta; z) = a, Z^{(r)}(0, \zeta; z) = 0$.

THEOREM. *For every rational r and complex a and ζ the formal power $Z^{(r)}(a, \zeta; z)$ exists and is uniquely determined.*

It is easy to see that $Z^{(r)}(a + b, \zeta; z) = Z^{(r)}(a, \zeta; z) + Z^{(r)}(b, \zeta; z)$, and that for every real α, $Z^{(r)}(\alpha a, \zeta; z) = \alpha Z^{(r)}(a, \zeta; z)$.

With the aid of the formal powers we may associate with every function $f(z)$ which is pseudo-analytic at ζ the "differential quotients"

$$D_\zeta^0 f = f(\zeta), \quad D_\zeta^n f = n! \lim_{z \to \zeta} \frac{\zeta^{(z)} \left\{ f(z) - \sum_{\nu=0}^{n-1} Z^{(\nu)}(D_\zeta^\nu f/\nu!, \zeta; z) \right\}}{(z - \zeta)^n},$$

$$n = 1, 2, \ldots.$$

These differential quotients always exist, though $f(z)$ considered as a function of x and y need not have partial derivatives of order higher than the second.

THEOREM. *If $f(z)$ is pseudo-analytic at ζ and $D_\zeta^n f = 0, n = 0, 1, \ldots,$ then $f(z) \equiv 0.$*

It is easy to see that the uniform limit f of a sequence of pseudo-analytic functions $\{f_j\}$ is pseudo-analytic. It can be shown that if $f_j \to f$ uniformly in a domain D, then $D_\zeta^\nu f_j \to D_\zeta^\nu f$, the convergence being uniform with respect to ζ in every compact subset of D.

5. *Rational Functions.*—We say that a pseudo-analytic function is a rational function if it has no singularities except poles, an entire rational function if it has no singularities except perhaps a pole at infinity.

THEOREM. *Every non-constant rational function has as many zeros as it has poles (provided poles and zeros are counted with proper multiplicities). Every rational function is a sum of formal powers with integral exponents. If $\zeta_i(i = 0, 1, \ldots, R)$ are distinct points, $n_i(i = 1, 2, \ldots, R)$ non-vanishing integers such that $n_1 + n_2 + \ldots + n_R = 0$ and b a non-vanishing complex number, then there exists one and only one rational function assuming at ζ_0 the value b, having at $\zeta_i(i = 1, 2, \ldots, R)$ a zero of order n_i if $n_i > 0$ and a pole of order $- n_i$ if $n_i < 0$, and having no other zeros or poles.*

If $f(z)$ is an entire rational function and ζ a given complex number, then $f(z)$ may be written as a "formal polynomial with center ζ":

$$f(z) = \sum_{\nu=0}^{n} Z^{(\nu)}(a_\nu, \zeta; z). \tag{2}$$

We say that this formal polynomial is of degree n if $a_n \neq 0$. It then has exactly n zeros.

THEOREM. *A formal polynomial of degree n is uniquely determined by its center, its leading coefficient and its n zeros. These parameters may be prescribed arbitrarily.*

Instead of prescribing the zeros of (2) we may require that $f(z)$ should satisfy the conditions $D_\zeta^\nu f = b_{\nu j}, \nu = 0, 1, \ldots, N_j, j = 1, 2, \ldots, R$, provided that $\zeta_j \neq \zeta_l$ for $j \neq l$, and $N_1 + N_2 + \ldots + N_R + R = n$.

6. *Formal Power Series and the Expansion Theorem.*—A formal power series is a series of the form

$$\sum_{\nu=0}^{\infty} Z^{(\nu)}(a_\nu, \zeta; z). \tag{3}$$

Together with (3) we consider the ordinary power series $\sum a_\nu s^\nu$ and denote its radius of convergence by r.

THEOREM. *The series (3) converges absolutely and represents a pseudo-analytic function for* $|s - \zeta| < r$, *converges uniformly for* $|s - \zeta| \leq r' < r$ *diverges for* $|s - \zeta| > r$.

A similar theorem holds for series of the form $\sum Z^{(-\nu)}$.

THEOREM. *Let $f(s)$ be pseudo-analytic for* $|s - \zeta| < R$ *and set $a_\nu = D_\zeta^\nu f/\nu!$. There exists a positive number α depending only on the function σ, such that the series (3) converges and represents the function $f(s)$ for* $|s - \zeta| < \alpha R$.

Thus if f is an entire function ($R = +\infty$), the series converges for all finite values of s. A similar expansion theorem holds for functions pseudo-analytic for $|s - \zeta| > R$.

7. *Cauchy's Formula.*—The second order equations resulting from (1) are

$$(u_x/\sigma)_x + (u_y/\sigma)_y = 0, \qquad (\sigma v_x)_x + (\sigma v_y)_y = 0. \tag{4}$$

Using the so-called fundamental solutions of these equations the values of u and v at an interior point of a domain may be expressed by line-integrals extended over the boundary of the domain and involving the boundary values of u and v (Bergman, Položii).

We are also able, however, without using explicitly the fundamental solutions, to obtain a Cauchy type formula formally identical with the classical one.

We note first that $Z^{(r)}(a, \zeta; s)$ depends continuously on a and ζ (at least for $\zeta \neq s$). Hence if C is a rectifiable arc admitting the parametric representation $\zeta = \zeta(s)$, $|\zeta'(s)| = 1$, $s_0 \leq s \leq s_1$, and $A(\zeta)$ a continuous function defined on C, the integral

$$\int_C Z^{(r)}[A(\zeta)d\zeta, \zeta; s] = \int_{s_0}^{s_1} Z^{(r)}\{A[\zeta(s)]\zeta'(s), \zeta; s\} ds$$

is defined for every s not on C.

THEOREM. *Let D be a domain bounded by a simple closed rectifiable curve C, $f(s)$ a function which is pseudo-analytic in D and continuous on C. Then*

$$\frac{1}{2\pi} \int_C Z^{(-1)}\{[^{r(r)}f(\zeta)]id\zeta, \zeta; s\} = f(s)$$

if s in an interior point of D. The integral vanishes if s is an exterior point of D.

We proceed to enumerate some consequences of this theorem.

8. *Isolated Singularities.*—THEOREM. *Let $f(s)$ be single-valued and pseudo-analytic for* $0 < |\zeta - s| < R$. *Then*

$$f(s) = \sum_{\nu = -\infty}^{+\infty} Z^{(\nu)}(a_\nu, \zeta; s),$$

the expansion being convergent for $0 < |\zeta - s| < \alpha R$.

Here a is the constant of the second theorem in §6. The number of non-vanishing $a_{-\nu}$, determines in the usual way the character of the point ζ.

9. *Approximation Theorem.*—Let D be a bounded domain bounded by the closed rectifiable curves C_0, C_1, ... C_N, C_0 being the outer boundary curve. Let ζ_0 be a point exterior to C_0 (which may be the point at infinity), ζ_j $(j = 1, 2, \ldots, N)$ a point interior to C_j. In D we consider a single-valued pseudo-analytic function $f(z)$.

THEOREM. *There exists a sequence of rational pseudo-analytic functions having no poles except at the points $\zeta_0, \ldots, \zeta_N$, which converges to $f(z)$ in D, the convergence being uniform in every compact subset of D.*

COROLLARY. *If D is simply connected, f may be expanded in a series of formal polynomials.*

Recently Eichler[13] established the following theorem for certain linear partial differential equations of elliptic type in n-space with analytic co-efficients. Let D_1, D_2, D_3 be bounded domains homeomorphic to the interior of the n-sphere, such that $\overline{D}_1 \subset D_2, \overline{D}_2 \subset D_3$, $\overline{D}$ denoting the closure of D. Let φ be a solution of the equation considered defined in D_2 and ϵ a positive number. Then there exists a solution ψ defined in D_3 such that $|\varphi - \psi| < \epsilon$ in D_1. A more special theorem of this kind has been obtained previously by Bergman.[14] Our result shows that for equations of the form (4) Eichler's theorem holds without analyticity assumptions.

10. *The Logarithmic Function.*—Let ζ' and ζ'' be two distinct points, and set

$$L(a, \zeta', \zeta''; z) = \int_{\zeta'}^{\zeta''} Z^{(-1)}\{[^{1/\sigma(\zeta)}a]d\zeta, \zeta; z\},$$

the integration being performed along some fixed path.

THEOREM. *The function $f(z) = L(a, \zeta', \zeta''; z)$ is pseudo-analytic for $z \neq \zeta', \zeta''$ and is such that the difference $f(z) - [a \log (z - \zeta') - a \log (z - \zeta'')]$ is single-valued and $O(|\log|(z - z')(z - z'')||)$. Any other function with these properties differs from $f(z)$ by a constant.*

From this theorem it is not difficult to infer the existence of a function $f(z) = L(a, \zeta, \infty; z)$ which is pseudo-analytic for $z \neq \zeta, \infty$ and differs from $a \log (z - \zeta)$ by a single-valued function $= O(|\log|z - z||)$. If a is real, then $u = \operatorname{Re} L(a, \zeta', \zeta''; z)$ is a fundamental solution of the first equation (4) for every domain containing ζ' but not ζ''.

11. *Multiple-Valued Functions.*—THEOREM. *Let $f(z)$ be a k-valued pseudo-analytic function defined for $0 < |\zeta - z| < r$. Then*

$$f(z) = \sum_{\nu = -\infty}^{+\infty} Z^{(\nu/k)}(a_\nu, \zeta; z),$$

the expansion being convergent at all points sufficiently near to and distinct from ζ.

If only a finite number of the coefficients $a_{-1}, a_{-2}, \ldots$, are different from

zero, we say that ζ is an algebraic singularity of $f(z)$. Algebraic singularities at $z = \infty$ are defined similarly. A function with only algebraic singularities is called algebraic.

THEOREM. *The sum of all determinations of an algebraic pseudo-analytic function is a pseudo-analytic rational function.*

If $f(z)$ is algebraic, there is associated with it a Riemann surface on which it is single-valued. The genus of this (closed) Riemann surface will be called the genus of $f(z)$.

THEOREM. *Let $f(z)$ be an algebraic pseudo-analytic function of genus p. Then it admits a parametric representation of the form*

$$z = \varphi(t), f = \omega(t)$$

where $\varphi(t)$ is a single-valued analytic function and $\omega(t)$ a single-valued function which is pseudo-analytic with respect to an admissible function $\rho(t)$. If $p = 0$, then $\rho(t)$ is admissible over the whole t-plane, and φ and ω are rational. If $p = 1$, ρ is admissible for $|t| < +\infty$ and all three functions ρ, φ, ω are doubly-periodic with the same periods. If $p > 1$, then ρ is admissible for $|t| < 1$, and all three functions ρ, φ, ω are automorphic with respect to the same group of hyperbolic motions.

While this theorem is almost self-evident (as is the corresponding uniformization theorem for non-algebraic functions) we state it in order to show how the investigation of multiple-valued solutions of (1) leads naturally to the theory of pseudo-analytic functions on Riemann surfaces. This theory should also include equations of the form (1) with multiple-valued $\sigma(x, y)$ and the case where $\sigma(x, y)$ becomes discontinuous or vanishes at certain points and on certain lines.

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THE EXCEPTIONAL SIMPLE LIE ALGEBRAS F_4 AND E_6

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Let K be an algebraically closed field of characteristic 0. The exceptional simple Jordan algebra $\mathfrak{J}$ over K is the (non-associative) algebra of dimension 27 whose elements are 3×3 Hermitian matrices

$$X = \begin{pmatrix} \xi_1 & x_3 & \bar{x}_2 \\ \bar{x}_3 & \xi_2 & x_1 \\ x_2 & \bar{x}_1 & \xi_3 \end{pmatrix} \tag{1}$$

with elements in the Cayley algebra $\mathfrak{C}$ of dimension 8 over K, multiplication being defined by

$$X \circ Y = \frac{1}{2}(XY + YX)$$

where XY is the ordinary matrix product. We write the trace

$$\xi_1 + \xi_2 + \xi_3 = Sp\, X.$$

The derivation algebra $\mathfrak{D}$ of $\mathfrak{J}$ is the Lie algebra of endomorphisms D of $\mathfrak{J}$ satisfying

$$D(X \circ Y) = DX \circ Y + X \circ DY. \tag{2}$$

By a right multiplication R_Y is meant the endomorphism

$$X \to X \circ Y \qquad \text{for every } X \text{ in } \mathfrak{J}.$$

We characterize the exceptional simple Lie algebras of dimension 52 and 78 over K by means of $\mathfrak{J}$ as follows:

THEOREM. *The exceptional simple Lie algebra F_4 of dimension 52 and rank 4 over K is the derivation algebra $\mathfrak{D}$ of the exceptional Jordan algebra $\mathfrak{J}$ of dimension 27 over K. The exceptional simple Lie algebra E_6 of dimension 78 and rank 6 over K is the Lie algebra*

$$\mathfrak{D} + \{R_Y\}, \qquad Sp\, Y = 0, \tag{3}$$

spanned by the derivations of $\mathfrak{J}$ and the right multiplications of elements Y of trace 0.

We begin by proving a Principle of Triality for the elements of the orthogonal Lie algebra $\mathfrak{o}(8, K)$ of all 8×8 skew-symmetric matrices over K. We regard the elements of $\mathfrak{o}(8, K)$ as endomorphisms of the Cayley algebra $\mathfrak{C}$. This algebra is equipped with a trace function $Sp\, x = x + \bar{x}$ satisfying

$$Sp \; xy \; = \; Sp \; yx, \qquad Sp \; x(yz) \; = \; Sp \; (xy)z \; (= \; Sp \; xyz).$$

Also $Sp \; xy$ is a non-degenerate bilinear form. The alternative law in $\mathfrak{C}$ gives the formula

$$Sp \; (sx)(yz) + Sp \; (ys)(zx) \; = \; Sp \; (xy)(sz + zs) \text{ for } x, y, z, s \text{ in } \mathfrak{C}. \quad (4)$$

For, writing $[x, y, z] = x(yz) - (xy)z$, we have $[x, y, z] = [z, x, y]$, so that $x(yz) + (zx)y = z(xy) + (xy)z$ and $Sp \; (sx)(yz) + Sp \; (ys)(zx) = Sp \; s(x(yz)) + Sp \; ((zx)y)s = Sp \; (x(yz) + (zx)y)s = Sp \; (z(xy) + (xy)z)s = Sp \; (xy)(sz + zs)$

An endomorphism U of $\mathfrak{C}$ is in $\mathfrak{o}(8, K)$ if and only if U leaves the norm form $x\bar{x}$ invariant:

$$(Ux)\bar{x} + x(\overline{Ux}) \; = \; 0. \quad (5)$$

The derivations D of $\mathfrak{C}$ satisfy (5); so do the left and right multiplications $L_s: x \to sx$ and $R_t: x \to xt$ of elements s and t of trace 0. It follows that every element U in $\mathfrak{o}(8, K)$ may be written uniquely in the form

$$U \; = \; D + L_s + R_t, \qquad \bar{s} = -s, \bar{t} = -t. \quad (6)$$

For if $D + L_s + R_t = 0$, then $t = -s$ and $L_s - R_s$ is a derivation of $\mathfrak{C}$: $s(yz) - (yz)s = (sy)z - (ys)z + y(sz) - y(zs)$ for all y, z in $\mathfrak{C}$. That is, $[s, y, z] + [y, z, s] - [y, s, z] = 3[s, y, z] = 0$, and s associates with all y, z in $\mathfrak{C}$. Then $s = 0, D = L_s = R_t = 0$. Since $\mathfrak{o}(8, K)$ is of dimension 28, while the derivation algebra of $\mathfrak{C}$ is the exceptional simple Lie algebra G_2 of dimension 14, every element of $\mathfrak{o}(8, K)$ is a sum (6).

PRINCIPLE OF TRIALITY. *For U in $\mathfrak{o}(8, K)$, there exist unique U', U'' in* $\mathfrak{o}(8, K)$ *such that*

$$Sp \; (Ux)yz + Sp \; x(U'y)z + Sp \; xy(U''z) \; = \; 0 \quad (7)$$

for all x, y, z in $\mathfrak{C}$.

For $U = D$, take $U' = U'' = D$. Then $0 = Sp \; D(xyz) = Sp \; (Dx)yz + Sp \; x(Dy)z + Sp \; xy(Dz)$. For $U = L_s$, take $U' = R_s$, $U'' = -L_s - R_s$. Then $Sp \; (sx)(yz) + Sp \; x(ys)z - Sp \; (xy)(sz + zs) = Sp \; (sx)(yz) + Sp \; (ys)(zx) - Sp \; (xy)(sz + zs) = 0$ by (4). Similarly for $U = R_t$, take $U' = L_t, U'' = -L_t - R_t$. But any U in $\mathfrak{o}(8, K)$ may be written in the form (6), so the existence of U', U'' satisfying (7) is assured. To show the uniqueness, we show that, if $Sp \; x(U'y)z + Sp \; xy(U''z) = 0$ identically, we have $U' = U'' = 0$. Since $Sp \; xy$ is a non-degenerate bilinear form, $Sp \; x((U'y)z + y(U''z)) = 0$ for all x, y, z implies

$$(U'y)z + y(U''z) \; = \; 0 \qquad \text{for all } y, z \text{ in } \mathfrak{C}. \quad (8)$$

Let $z = 1$ in (8); then $U'y = yu$ for u in $\mathfrak{C}$ with $Sp \; u = 0$. Let $y = 1$ in (8): then $U''z = vz$ for v in $\mathfrak{C}$. Hence (8) becomes

$$(yu)z + y(vz) = 0 \qquad \text{for all } y, z \text{ in } \mathfrak{C}. \tag{9}$$

Putting $y = z = 1$ in (9), we have $v = -u$, $[y, u, z] = 0$, or u associates with all y, z in C. Then $u = 0$, $U' = U'' = 0$.

Associated with the exceptional simple Jordan algebra $\mathfrak{J}$ are the bilinear form $Sp\,X \cdot Y$ and the trilinear form

$$\phi(X, Y, Z) = Sp\,(X \cdot Y) \cdot Z = Sp\,X \cdot (Y \cdot Z).$$

Any derivation D of $\mathfrak{J}$ leaves these forms invariant:

$$Sp\,DX \cdot Y + Sp\,X \cdot DY = 0, \tag{10}$$

$$\phi(DX, Y, Z) + \phi(X, DY, Z) + \phi(X, Y, DZ) = 0. \tag{11}$$

Conversely, any endomorphism D of $\mathfrak{J}$ leaving both $Sp\,X \cdot Y$ and $\phi(X, Y, Z)$ invariant is a derivation of $\mathfrak{J}$. For (10) implies $Sp\,(D(X \cdot Y)) \cdot Z + \phi(X, Y, DZ) = 0$. With (11) this gives

$$Sp\,(DX \cdot Y + X \cdot DY - D(X \cdot Y)) \cdot Z = 0 \tag{12}$$

identically. Since $Sp\,X \cdot Y$ is a non-degenerate bilinear form, (12) implies (2), D is a derivation of $\mathfrak{J}$.

Denote by e_i the matrix (1) with $\xi_i = 1$, all remaining entries zero. Let $\mathfrak{T}_i$ be the set of matrices (1) with all entries except x_i and $\bar{x}_i$ zero; $\mathfrak{T}_i$ is then in a natural one-to-one correspondence with $\mathfrak{C}$. If $T_i \in \mathfrak{T}_i$, then $e_i \cdot T_i = 0$, $e_j \cdot T_i = \frac{1}{2}T_i$ if $j \neq i$. We propose first to determine all derivations which map e_1, e_2 and e_3 upon 0. The spaces $\mathfrak{T}_i$ are invariant under such a derivation D, since $0 = D(e_i \cdot T_i) = (De_i) \cdot T_i + e_i \cdot (DT_i) = e_i \cdot (DT_i)$ implies DT_i is in $\mathfrak{T}_i + Ke_j + Ke_k$ where $\{i, j, k\} = \{1, 2, 3\}$, while $\frac{1}{2}DT_i = D(e_j \cdot T_i) = (De_j) \cdot T_i + e_j \cdot (DT_i) = e_j \cdot (DT_i)$ implies DT_i is in $\mathfrak{T}_i + \mathfrak{T}_k$. Let U be the restriction of D to $\mathfrak{T}_1$. Since D leaves invariant the quadratic form

$$Sp\,X^2 = \sum \xi_i^2 + 2\sum x_i \bar{x}_i,$$

we see that U leaves invariant the norm form $x_1 \bar{x}_1$, so U is in $\mathfrak{o}(8, K)$. Also the restrictions U' and U'' of D to $\mathfrak{T}_2$ and $\mathfrak{T}_3$ are in $\mathfrak{o}(8, K)$. But since D leaves invariant the cubic form

$$Sp\,X^3 = \sum \xi_i^3 + 2\sum \xi_i(x_j \bar{x}_j + x_k \bar{x}_k) + 3Sp\,x_1 x_2 x_3,$$

it follows that U, U', U'' satisfy (7). Hence any derivation of $\mathfrak{J}$ which maps e_1, e_2, e_3 upon 0 has the form

$$X \rightarrow \begin{pmatrix} 0 & U''x_3 & U'\bar{x}_2 \\ U''\bar{x}_3 & 0 & Ux_1 \\ U'x_2 & U\bar{x}_1 & 0 \end{pmatrix} \tag{13}$$

for X in (1), where U is in $\mathfrak{o}(8, K)$ and U', U'' are the corresponding elements of $\mathfrak{o}(8, K)$ given by the Principle of Triality. Conversely, any mapping (13) is a derivation of $\mathfrak{J}$ since it leaves invariant both $Sp\ X \bullet Y$ and $\phi(X, Y, Z)$. The mappings (13) then give 28 linearly independent derivations of $\mathfrak{J}$, and actually form a subalgebra of $\mathfrak{D}$ isomorphic to $\mathfrak{o}(8, K)$.

The space of all elements of trace 0 of $\mathfrak{J}$ which are mapped upon 0 by R_{e_i} is the space $\mathfrak{X}_i' = \mathfrak{X}_i + K(e_j - e_k)$. Let $\mathfrak{D}_i$ be the space of derivations of $\mathfrak{J}$ which map e_i upon 0; then the elements of $\mathfrak{D}_i$ map $\mathfrak{X}_i'$ into itself and leave invariant the restriction to $\mathfrak{X}_i' \times \mathfrak{X}_i'$ of the bilinear form $Sp\ X \bullet Y$. On the other hand, the only operation of $\mathfrak{D}_i$ which maps $\mathfrak{X}_i'$ upon $\{0\}$ is 0; for such an operation maps e_i, $e_j - e_k$, and $e_i + e_j + e_k$ upon 0, which shows that it belongs to $\mathfrak{o}(8, K)$, and therefore that it is 0 since it maps $\mathfrak{X}_i$ upon 0. The algebra $\mathfrak{D}_i$ is therefore isomorphic with a subalgebra of $\mathfrak{o}(9, K)$. On the other hand, if X and Y are in $\mathfrak{J}$, then

$$[R_X, R_Y] = R_X R_Y - R_Y R_X$$

is a derivation, because $\mathfrak{J}$ is a Jordan algebra.[1] In particular, if $T_1 \in \mathfrak{X}_1$, the operation $[R_{T_1}, R_{e_2 - e_3}]$ is in $\mathfrak{D}_1$ and maps $e_2 - e_3$ upon T_1. Since the operations of $\mathfrak{o}(8, K)$ map $e_2 - e_3$ upon 0, we have dim $\mathfrak{D}_1 \geq 28 + 8 = 36 = $ dim $\mathfrak{o}(9, K)$, and $\mathfrak{D}_1$ is isomorphic with $\mathfrak{o}(9, K)$; the same is of course true of $\mathfrak{D}_2$ and $\mathfrak{D}_3$.

Let D be any derivation of $\mathfrak{J}$. Then $De_1 = De_1^2 = 2e_1 \circ De_1$, whence $De_1 = T_2 + T_3 \in \mathfrak{X}_2 + \mathfrak{X}_3$. We can find operations D_2 and D_3 of $\mathfrak{D}_2$ and $\mathfrak{D}_3$, respectively, such that $D_2 e_1 = T_2$, $D_2 e_1 = T_3$, and D_2 and D_3 are then uniquely determined modulo $\mathfrak{o}(8, K)$. The operation $D - (D_2 + D_3)$ is then in $\mathfrak{D}_1$; this proves that the algebra $\mathfrak{D}$ of derivations of $\mathfrak{J}$ is $\mathfrak{D}_1 + \mathfrak{D}_2 + \mathfrak{D}_3$ and that its dimension is $(36 - 28) + (36 - 28) + 36 = 52$. The adjoint representation of $\mathfrak{D}$ induces a representation of $\mathfrak{o}(8, K)$ which is the sum of the adjoint representation and of the representations given by the spaces $\mathfrak{D}_i/\mathfrak{o}(8, K)$ $(i = 1, 2, 3)$. If we map every $D \in \mathfrak{D}_i$ upon $D(e_j - e_k)$ (where (i, j, k) is an even permutation of $(1, 2, 3)$), then we obtain equivalences of the representations given by the spaces $\mathfrak{D}_1/\mathfrak{o}(8, K)$, $\mathfrak{D}_2/\mathfrak{o}(8, K)$, $\mathfrak{D}_3/\mathfrak{o}(8, K)$ with the representations $U \to U$, $U \to U'$, and $U \to U''$ constructed above, and it is easily seen that these representations are inequivalent to each other and to the adjoint representation of $\mathfrak{o}(8, K)$. Thus the space $\mathfrak{D}$, considered as a representation space of $\mathfrak{o}(8, K)$, splits into four inequivalent simple representation spaces. It follows that any ideal $\mathfrak{a}$ in $\mathfrak{D}$ must be the sum of 0, 1, 2, 3 or 4 of these spaces. On the other hand, each $\mathfrak{D}_i$ being simple, the intersection of an ideal with $\mathfrak{D}_i$ is either $\{0\}$ or $\mathfrak{D}_i$. It follows immediately that the only ideals in $\mathfrak{D}$ are $\{0\}$ and $\mathfrak{D}$; that is, $\mathfrak{D}$ is simple. Since there is only one simple Lie algebra of dimension 52, namely F_4, we see that $\mathfrak{D}$ is the algebra F_4. The set $\mathfrak{J}_0$ of elements of trace 0 in $\mathfrak{J}$ is mapped into itself by the operations of $\mathfrak{D}$ and yields a representation of de-

gree 26 of $\mathfrak{D}$. Considered as a representation space of $o(8, K)$, $\mathfrak{J}_0$ splits into two invariant spaces of dimension 1 (spanned by $e_1 - e_2$ and $e_1 - e_3$, respectively) and three inequivalent spaces of dimension 8 (namely, $\mathfrak{X}_1$, $\mathfrak{X}_2$ and $\mathfrak{X}_3$). Any invariant subspace of $\mathfrak{J}_0$ with respect to $\mathfrak{D}$ of dimension > 2 must therefore contain one of the spaces $\mathfrak{X}_i$. If it contains $\mathfrak{X}_1$, then, being invariant with respect to $\mathfrak{D}_1$, it contains $e_2 - e_3$, and, being invariant with respect to $\mathfrak{D}_2$ and $\mathfrak{D}_3$, it contains $\mathfrak{X}_2$ and $\mathfrak{X}_3$. It follows immediately that $\mathfrak{J}_0$ gives a simple representation of $\mathfrak{D}$.

If X and Y are in $\mathfrak{J}$, then $[R_X, R_Y]$ is in $\mathfrak{D}$; moreover, if $D \in \mathfrak{D}$, then $[D, R_Y] = R_{DY}$ and $Sp \, DY = 0$. It follows that (3) is a Lie algebra $\mathfrak{E}$. Since $D(1) = 0$ for every derivation D of $\mathfrak{J}$, $D + R_Y = 0$ implies $Y = 0$, $D = 0$; thus $\mathfrak{E}$ is of dimension 78, and the adjoint representation of $\mathfrak{E}$ induces a representation of $\mathfrak{D}$ which is the sum of the adjoint representation and of the representation whose space is the set $\mathfrak{R}$ of right multiplications of elements in $\mathfrak{J}_0$. Since $\mathfrak{D}$ and $\mathfrak{R}$ yield simple representation spaces of $\mathfrak{D}$ of distinct dimensions, the only possible ideals in $\mathfrak{E}$ are $\{0\}$, $\mathfrak{D}$, $\mathfrak{R}$ and $\mathfrak{D} + \mathfrak{R} = \mathfrak{E}$; but $\mathfrak{D}$ and $\mathfrak{R}$ are obviously not ideals, which proves that $\mathfrak{E}$ is a simple Lie algebra. The space $\mathfrak{J}$ yields an obviously simple representation of degree 27 of $\mathfrak{E}$. Now, outside E_6, the only simple Lie algebras of dimension 78 are the symplectic algebra $\mathfrak{p}(12, K)$ and the orthogonal algebra $o(13, K)$. Using the formulas of H. Weyl for the computation of the degrees of the simple representations,[2] we find that the degree of the simple representation of $\mathfrak{p}(12, K)$ of smallest degree > 12 is 65, while the degree of the simple representation of degree > 13 of $o(13, K)$ is 64. It follows that $\mathfrak{E}$ is the exceptional simple algebra E_6.

[1] Albert, A. A., "A Structure Theory for Jordan Algebras," *Ann. Math.*, **48**, 550, formula (8) (1947).

[2] Weyl, H., *The Classical Groups*, Chap. VII, §§ 8 and 9. Actually Weyl gives here the explicit formula only for the degrees of the representations of the symplectic group, but the formula for the orthogonal group can be obtained in exactly the same manner.

A TOPOLOGIZED FUNDAMENTAL GROUP[1]

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Introduction.—For a $C^\circ LC^1$ Hausdorff space, it is well known that there is a 1-1 correspondence between its covering spaces and the subgroups of its fundamental group.[2] This note arose from an investigation to determine whether non-trivial[3] covering spaces for $C^\circ LC^\circ$ spaces can be constructed, and whether they can be classified in some fashion. Our principal tool is the topologized fundamental group of Hurewicz.[4]

1. The Group χ.—Instead of continuous deformations, we use step-homotopies. Let Y be a topological space and σ a covering of Y by open sets. The path[5] α_1 is σ-homotopic to the path α_n, $\alpha_1 \sigma \simeq \alpha_n$, if there exists a finite sequence $\alpha_1, \ldots, \alpha_n$ of paths, all with the same beginning and ending points, such that for every t, $0 \leq t \leq 1$ and each $i = 1, 2, \ldots, n - 1$, $\alpha_i(t)$ and $\alpha_{i+1}(t)$ are contained in a common set of σ. Two paths are equivalent if they are σ-homotopic for every σ. With the usual definition of product and inverse of paths, the set of all equivalence classes of paths closed at a point $y_0 \in Y$ forms a group. Denote the equivalence class of α by $\underline{\alpha}$. $\chi(Y, y_0)$ is this group, with the topology: given a covering σ of Y, the σ-*nbd* of α, $N(\alpha, \sigma) = \{\beta | \beta \sigma \simeq \alpha\}$; the sets $N(\underline{\alpha}, \sigma)$ are taken as a basis.[6]

1. $\chi(Y, y_0)$ is a topological group which (a) is zero dimensional, (b) has arbitrary small open normal subgroups, (c) is metrizable, and (d) is completeable.[7] If Y is C°, the collection of groups $\{\chi(Y, y_0)\}$ is a system of local groups.[8]

The position of χ relative to the Poincaré group π_1 and the Vietoris fundamental group[9] V_1 is given by

2. If Y is C° and $\chi = \pi_1$, then Y is[10] ω-LC_1. If Y is a $C^\circ LC^\circ \omega LC_1$ space with barycentric refinements,[11] then $\chi = \pi_1$. In a compact $C^\circ LC^\circ$ metric space, V_1 is homeomorphic with the completion of χ.

An $F:X \to Y$ yields a continuous homomorphism $F_+ : \chi(X, x_0) \to \chi(Y, F(x_0))$ induced by the correspondence $\alpha : F\alpha$; F_+ is a homotopy invariant.

2. Open Subgroups and Coverings.—Let Y be C° and $\mathcal{K}$ any open subgroup of $\chi(Y, y_0)$; choose a covering σ so that $N(1, \sigma) \subset \mathcal{K}$. Two paths α, β starting at y_0 and ending at any $y \in Y$ are equivalent mod $\mathcal{K}$ if $\underline{\alpha\beta^{-1}} \in \mathcal{K}$; the equivalence class of α is written $[\alpha]$. These equivalence classes, for all $y \in Y$ are the points of $\tilde{Y}$. Given α and any open $U \supset \alpha(1)$, U contained in some set of σ, define $(\alpha, U) = \{[\alpha\beta] | \beta \subset U\}$; the sets (α, U) are taken as a basis in $\tilde{Y}$. The projection mapping $\omega : \tilde{Y} \to Y$ is defined by $\omega\{\alpha\} = \alpha(1)$. Y is a Hausdorff space, and

3. Let Y be C°, $\tilde{Y}$ constructed as above. Then $\tilde{Y}$ is C°, ω is continuous, and ω induces a continuous 1-1 mapping of[12] $\tilde{Y}^1(\tilde{y})$ onto $Y^1(\omega\tilde{y})$ for each $\tilde{y} \in \tilde{Y}$. Closed paths at $\tilde{y}_0$ are sent on all those, and on only those closed paths at $\omega\tilde{y}_0 = y_0$ representing elements of $\mathcal{K}$.

Adding an LC° assumption, $\tilde{Y}$ is a covering space, $\chi(\tilde{Y}, \tilde{y}_0) = \mathcal{K}$ and we have

THEOREM. *If Y is $C^\circ LC^\circ$ Hausdorff, its covering spaces are in a 1-1 correspondence with the open subgroups of χ. Precisely: (a) if $\tilde{Y}$ is any covering space of Y, then $\chi(\tilde{Y})$ is an open subgroup of $\chi(Y)$; (b) if $\mathcal{K}$ is any open subgroup of $\chi(Y)$, there exists a covering space with $\chi(\tilde{Y}) = \mathcal{K}$, and (c) any two coverings with homeomorphic χ's are themselves homeomorphic.*

· In general then, universal covering spaces[13] for $C^\circ LC^\circ$ spaces do not exist.

3. *Closed Subgroups.*—With a closed subgroup $B \subset \chi(Y)$ construct a $\bar{Y}$ as follows: the elements and the projection map are defined as in 2. Topology: for any covering σ, and open $U \in \sigma$, define $(\alpha, U, \sigma) = \{[\beta]|\beta(1) \in U,$ and $\exists$ a $\delta \subset U$ with $\beta\sigma \simeq \alpha\delta\}$. The sets (α, U, σ) are taken as a basis. $\bar{Y}$ is a Hausdorff space, and

4. If Y is C°, $\bar{Y}$ as above, then every fiber $\omega^{-1}(y)$ is homeomorphic to the coset space χ/B.

It follows that for any closed B, χ/B is always totally arcwise disconnected.

In addition we have the analog of 3.

5. Let Y be C° and $\bar{Y}$ as above. Then $\bar{Y}$ is C°, ω is continuous, and ω induces a continuous 1-1 mapping of $\bar{Y}^I(\bar{y})$ onto $Y^I(\omega\bar{y})$ for every $\bar{y} \in Y$. Closed paths at $\bar{y}_0$ are sent on all those and on only those closed paths at $\omega\bar{y}_0 = y_0$ representing elements of B. If Y is $C^\circ LC^\circ$, this correspondence is a homeomorphism.

Even with the LC° assumption, $\bar{Y}$ is in general not even a fiber space;[14] however a covering homotopy theorem does hold in the LC° case. The calculation of $\chi(\bar{Y})$ is not known, except that in general, $\chi(\bar{Y}) \neq B$. This also shows that in Y^I, when Y is $C^\circ LC^\circ$, the set of closed paths at $y_0 \in Y$ equivalent to 1 is in general *not* a connected set, in contrast with the result that in Y^I the set of closed paths at $y_0 \in Y$ homotopic to 1 is arcwise connected.

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[1] This represents work done mainly under contract N7 onr-384, Office of Naval Research.

[2] See, e.g., Hopf, H., *Math. Ann.*, 102, 562-623 (1930). For the symbols C^n, LC^n, Kuratowski, C., *Fund. Math.*, 24, 269-287 (1934).

[3] Y is always a covering space of itself.

[4] Hurewicz, W., *Fund. Math.*, 25, 467-485 (1935).

[5] A path in Y is a continuous map of $0 \leq t \leq 1$ into Y.

[6] If Y is compact metric, χ is the Hurewicz group, *loc. cit.*

[7] In the sense of van Dantzig, D., *Math. Ann.*, 107, 587-626 (1932).

[8] Steenrod, N., *Ann. Math.*, 44, 610-627 (1943).

[9] Vietoris, L., *Math. Ann.*, 97, 545-572 (1928).

[10] Y is ω-LC_1 if there exists a covering σ of Y by open sets such that every closed path lying entirely in a set of σ is contractible to a point in Y.

[11] Y has barycentric refinements if, given any covering $\{U_\alpha\}$, a refinement $\{V_\beta\}$ exists with the property that, if $\bigcap V_{\beta\lambda} \neq 0$, then $\bigcup V_{\beta\lambda} \subset U_\alpha$ for some α.

[12] $Y^I(y)$ is the space of all paths in Y which start at y, with the compact open topology. Fox, R., *Bull. Am. Math. Soc.*, 51, 429-432 (1945).

[13] A universal covering space is one with $\chi(Y) = 1$. Clearly $\pi_1(Y) = 1$ implies $\chi(Y) = 1$.

[14] Hurewicz, W., and Steenrod, N., PROC. NAT. ACAD. SCI., 27, 60-64 (1941).

ON A GENERALIZATION OF A JACOBI EXPONENTIAL SUM ASSOCIATED WITH CYCLOTOMY

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Let $\alpha = e^{2i\pi/(p-1)}$, $\zeta = e^{2i\pi/p}$, p prime then the resolvent used by Lagrange[1] in his theory of the solution of cyclotomic equations, dating from the year 1770,

$$\sum_{a=1}^{p-2} \alpha^{c \text{ ind } a} \zeta^{ak} = \tau(\alpha^c, \zeta^k); \tag{1}$$

(ind a) is defined by $g^{\text{ind } s} \equiv s \pmod{p}$, g being a primitive root of p, was the starting point for a series of investigations which are still being carried on.

Closely associated (cf. (13)) with (1) is[2]

$$\psi_1(\alpha) = \sum_{k=0}^{p-2} \alpha^{-bh + (a+b)\text{ind }(g^h+1)}, \tag{2}$$

$$\alpha^{(a+b)\text{ ind }(0)} = 0,$$

with a, b and c any integers, which has probably been employed even oftener than (1) in algebra and number theory. In view of the remarkable properties of (1) and (2), a number of generalizations have been proposed for them.

Cauchy[2a] generalized (2) to take on a form equivalent to the sum (11) or (22) of the present paper if we assume $n = 1$ in each of them. These two latter forms for n general provide the main topic for our work here.

In the year 1920, H. H. Mitchell[3] considered, as an extension of (2)

$$\psi(\alpha) = \sum_{k=0}^{p^n-2} \alpha^{-bk + (a+b)\text{ ind }(g^k+1)}, \tag{3}$$

where ind s is defined by the equation $g^{\text{ind } s} = s$ in the finite field $F(p^n)$ of order p^n, p an odd prime and n an arbitrary integer > 0, s being any element of $F(p^n) \neq 0$, $\alpha^{\mu \text{ ind }(0)} = 0$ for μ any integer. An equivalent expression was used by Kummer[4] in the case where b and $(a+b)$ are divisible by the integer $(p^n - 1)/l$ and p belongs to the exponent l, where l is an odd prime.

Davenport and Hasse[5] in 1935 generalized (1) in the form

$$\tau(\alpha^\mu, \zeta^\nu) = \sum_a \chi^\mu(a)\zeta^{\nu \text{ tr }(a)}, \tag{4}$$

where now a ranges over all the elements of $F(p^n)$, $\mu \not\equiv 0 \pmod{m}$, $\nu \not\equiv 0 \pmod{p}$, $\chi(a)$ represents a character defined by the multiplicative cyclic group formed by the non-zero elements in $F(p^n)$, a ranging as stated, with

$\chi^{\mu}(0) = 0$, $\mu \neq 0$, and $\chi^{\mu}(0) = 1$, $\mu = 0$. The symbol $tr(a)$ means that if we write in $F(p^{\pi})$, with the c's in $F(p)$

$$a = c_0 + c_1\omega + \ldots + c_{\pi - 1}\omega^{\pi - 1},$$

where ω is a root of a polynomial $f(x) = 0$ of degree n with coefficients in $F(p)$, also irreducible in $F(p)$ then

$$tr(a) = \sum_{i=1}^{\pi} (c_1 + c_2\omega^{(i)} + \ldots + c_{\pi - 1}\omega^{(\pi - 1)(i)}),$$

where $\omega^{(1)} = \omega$, $\omega^{(2)}$, $\ldots$ $\omega^{(\pi)}$ are the n conjugate roots of $f(x) = 0$ in $F(p^{\pi})$. The r number of (4) will be termed a *Generalized Lagrange Resolvent.*

In the case where p belongs to the exponent n, modulo m, where α^{μ} is an mth root of unity, a number equivalent to (4) was introduced and studied at length by Stickelberger[6] in 1890 in a celebrated paper.

In a recent article, Hua and Vandiver employed[7] (4) in examining the number of solutions of the equation

$$c_1x_1^{a_1} + c_2x_2^{a_2} + \ldots + c_sx_s^{a_s} + c_{s+1} = 0, \tag{5}$$

where the a's are integers such that $0 < a < p^{\pi} - 1$; $s \geqq 2$ for $c_{s+1} \neq 0$ and $s > 2$ for $c_{s+1} = 0$ the c's being given elements of $F(p^{\pi})$ and

$$c_1c_2\ldots c_sx_1\ldots x_s \neq 0. \tag{5a}$$

If N is the number of solutions of (5) under the conditions just stated then they found

$$N = \frac{(p^{\pi} - 1)^s}{p^{\pi}} + \frac{T}{p^{\pi}}, \tag{6}$$

where

$$T = \sum_{\substack{a \cdot k \\ a \neq 0}} \prod_{i=1}^{s} \sum_{x_{k_i}} \chi_{k_i}(c_i^{-1}a^{-1})\tau(\chi_{k_i})\zeta^{tr(ac_{s+1})}. \tag{6a}$$

Here

$$\tau(\chi_{k_i}) = \sum_{b \cdot K} \chi_{k_i}(b)\zeta^{tr(b)}$$

where $\chi_k(b)$ is a special k_ith character each element of which satisfies $x^k = 1$, $p^{\pi} - 1 \equiv 0 \pmod{k_i}/k_i = (a_i, p^{\pi} - 1)$, K stands for $F(p^{\pi})$. In another recent paper, Weil[8] examined the number of solutions M of (5) *without the restriction, included in our* (5a), *that*

$$x_1x_2\ldots x_s \neq 0.$$

If $c_{s+1} = 0$ then in our notation he obtained if $r_i \not\equiv 0 \pmod{k_i}$,

$$M = p^{n(s-1)} + (p^n - 1)\sum_{\chi_{k_i}}\prod_{i=1}^{s}\chi_{k_i}(c_i^{-1})j(\alpha) \qquad (7)$$

where if $\psi_{k_i}(b) = \theta^{2i\pi r_i}/_{k_i}$ then $\sum_i r_i/_{k_i} \equiv 0 \pmod 1$,

$$j(\alpha) = \sum_{v_i}\chi_{k_i}(v_1)\ldots\chi_{k_s}(v_s) \qquad (7a)$$

and where $v_1, v_2, \ldots v_s$ each range independently over all elements of $F(p^n)$ such that $1 + v_1 + \ldots + v_{s-1} = 0$, ((7a) was given in another form by Cauchy[2a] for $n = 1$ and by (11) of the present paper for n general).

He also obtained an analogous expression for M when $c_{s+1} \neq 0$.

If we include the case where $x_1x_2\ldots x_s = 0$ we may obtain a form analogous to our (6) by using[7] (the relation just above (11) of that paper with the restriction $x \neq 0$ omitted).

$$\sum_{z \in K}\zeta^{tr(cz^k)} = \sum_{y \in k}\sum_{\chi_k}\chi_k(c^{-1}y)\zeta^{tr(y)}$$

where we now define $\chi_k(0)$ as in connection with (4), noting that there we are expressing each of the $\chi_k(a)$, a fixed, as a power of a primitive one of them. This gives if N_1 is the number of solutions of (5) without the restriction

$$x_1x_2\ldots x_s \neq 0$$

$$N_1 = p^{n(s-1)} + \frac{T_1}{p^n} \qquad (8)$$

where

$$T_1 = \sum_{a \in K}\prod_{i=1}^{s}\sum_{\chi_{k_i}}(c_i^{-1}a^{p^n-2})\tau(\chi_{k_i})\zeta^{tr(aa_{s+1})}$$

Our object in the present paper will be to develop some of the simplest properties of $j(\alpha)$ as given in (7a), without particular reference to the solution of equations in a finite field, except in connection with the proof of (15) below. We shall have occasion to employ these properties in subsequent papers.

A question of notation here assumes some importance. We may retain the notation of characters $\chi_k(w)$ in dealing with $j(\alpha)$ but since we are dealing with a *cyclic* group it seems more convenient to employ in place of $\chi_k(w)$ the explicit expression

$$\rho^{\text{ind } w} \qquad (9)$$

where ρ is a primitive kth root of unity and (ind w) is defined as in (3). *If we adopt the latter, we immediately connect up our work to many results in the*

vast literature of cyclotomy.   For example, Cauchy (who used $I(a)$ for ind a), Kummer, Kronecker, Weber, Bachmann, Mertens and Landau, among others, used it.   Also the fundamental property of characters

$$\chi(h_1)\chi(h_2) = \chi(h_1 h_2)$$

is at all times in the foreground when we transform expressions of the type (9), since

$$\text{ind } h_1 + \text{ind } h_2 \equiv \text{ind } h_1 h_2 (\text{mod } (p^n - 1)). \tag{10}$$

We shall employ (9) in the present paper.   From what we have just explained it will be easy to translate any of our formulas into the notation of characters if so desired.

We then consider the exponential sum

$$\psi = \psi(\alpha_1^{\mu_1}, \ldots, \alpha_s^{\mu_s}) = \sum_{a_1, a_2, \ldots, a_s} \alpha_s^{\mu_s \text{ ind } A} \prod_{i=1}^{s-1} \alpha_i^{\mu_i \text{ ind } a_i} \tag{11}$$

where $\alpha_i = \varepsilon^{2i\pi/m_i}$, $i = 1, 2, \ldots, s$; m_i is a divisor of $p^n - 1$; p an odd prime; μ_i, $i = 1, 2, \ldots s$, integers ≥ 0; ind a_i for $a_i \neq 0$ is defined by $g^{\text{ind } a_i} = a_i$ in $F(p^n)$, $i = 1, 2, \ldots s,$; g is a primitive root of $F(p^n)$; $\alpha_i^{\mu_i \text{ ind } (0)} = 0$; each a_i ranges independently over each element of $F(p^n)$, with

$$A = 1 - \sum_{i=1}^{s-1} a_i. \tag{11a}$$

The number ψ will be termed a *generalized Jacobi sum.*   When (12a) holds m_i) it equals the $j(\alpha)$ of Weil.   It was proved by Weil[8] (pp. 501–502) that

$$\psi\bar{\psi} = p^{n(s-2)} \tag{12}$$

provided

$$\left. \begin{array}{l} \alpha_i^{\mu_i} \neq 1, \ i = 1, 2, \ldots, s \\[4pt] \prod_{i=1}^{s} \alpha_i^{\mu_i} = 1. \end{array} \right\} \tag{12a}$$

It was also shown by Weil[8] (p. 501;   that for $\alpha_i^{\mu_i} \neq 1$; $i = 1, \ldots s,$

$$\psi = \frac{\prod_{i=1}^{s} \tau(\alpha_i^{\mu_i})}{p^n}, \tag{13}$$

provided $\alpha_1^{\mu_1}\alpha_2^{\mu_2}\ldots\alpha_s^{\mu_s} = 1$, where $\tau(\alpha_i^{\mu_i}) = \Sigma_a \alpha_i^{\mu_i \text{ ind } a} \zeta^{tr(a)}$.   Since for $a \neq 0$ in $F(p^n)$, there is an h such that $g^h = a$, we examine the following sum, which equals ψ or possibly its negative (cf. the derivation of our relation (24) which follows),

$$\psi_1 = \sum_{h_1,\ h_2,\ldots h_s} \alpha_s^{\mu_s \text{ ind } H} \prod_{i=1}^{s-1} \alpha_i^{\mu_i h_i}, \tag{14}$$

with

$$H = -1 - g^{h_1} - g^{h_2} \ldots - g^{h_{s-1}}.$$

We now proceed to write (14) in another more symmetrical form, namely

$$\psi_1 = \sum_{j_1,\ j_2,\ldots j_s} (j_1, j_2, \ldots, j_s) \prod_{i=1}^{s} \alpha_i^{\mu_i j_i}, \tag{15}$$

where $(j_1, j_2, \ldots, j_s)$ is the number of solutions of

$$1 + g^{j_1 + m_1 \gamma_1} + g^{j_2 + m_2 \gamma_2} \ldots + g^{j_s + m_s \gamma_s} = 0 \tag{16}$$

in the γ's, where γ_i ranges over the integers $0, 1, \ldots, m_i'$ with $m_i m_i' = p^n - 1$, and $i = 1, 2, \ldots, s$. To show that (14) and (15) are equal, we consider (14) and collect the terms in a certain way. Consider the residues of h_i modulo m, and write for fixed j_i $h_i = j_i (\text{mod } m_i)$; $i = 1, 2, \ldots s - 1$. Also assume that for a fixed j_s that ind $(-1 - g^{h_1} \ldots - g^{h_{s-1}}) \equiv j_s \,(\text{mod}\, (p^n - 1))$. The number of terms of this character in (14) is exactly $(j_1, j_2, \ldots, j_s)$ hence we have (15). From this it is obvious that we have for a fixed set $j_1', j_2', \ldots j_s'$, using $\sum_{\mu_i} \alpha_i^{k\mu_i} = 0$ for $k \neq 0 \,(\text{mod } m_i)$ and m_i otherwise

$$(j_1', j_2', \ldots j_s') \prod_{i=1}^{s} m_i = \sum_{\mu_1, \ldots \mu_s} \psi_1(\alpha_1^{\mu_1}, \alpha_2^{\mu_2} \ldots \alpha_s^{\mu_s}) \prod_{i=1}^{s} \alpha_i^{-\mu_i j_i'}, \tag{17}$$

where μ_i ranges over $0, 1, \ldots, (m_i - 1)$ with $i = 1, 2, \ldots s$.

We note that if

$$\tau^{p^n - 1} = \left(\sum \alpha^{s \text{ ind } a} \zeta^a\right)^{p^n - 1},$$

then this number is unaltered by the substitution (ζ / ζ^k) hence it contains α only.. Also it is known that we have

$$\tau(\alpha^{p^t}) = \tau(\alpha). \tag{18}$$

Further we may show that

$$\psi(\alpha_1^{\mu_1 p^t}, \alpha_2^{\mu_2 p^t}, \ldots, \alpha_s^{\mu_s p^t}) = \psi(\alpha_1^{\mu_1}, \ldots, \alpha_s^{\mu_s}). \tag{19}$$

For we note that if

$$\beta_1, \beta_2 \ldots \beta_d, \text{ with } p^n - 1 = d, \tag{20}$$

are the elements $\neq 0$ of a finite field then

$$\beta_1^{p^t}, \beta_2^{p^t} \ldots \beta_d^{p^t} \tag{21}$$

are equal in some order to the elements in (20) since if

$$\beta_i{}^{p^t} = \beta_j{}^{p^t};$$

$$(\beta_i{}^{p^t})^{p^{n-t}} = (\beta_j{}^{p^t})^{p^{n-t}}, \qquad \beta_i = \beta_j,$$

hence

$$\sum_{a_1, a_2, \atop a_s} \alpha_s{}^{p^t \mu_s \text{ ind } (A)} \prod_{i=1}^{s-1} \alpha_i{}^{p^t \mu_i \text{ ind } a_i}$$

$$= \sum_{a_1, a_2, \atop a_s} \alpha^{p^t \mu_s \text{ ind } B} \prod_{i=1}^{s-1} \alpha_1{}^{\mu_i \text{ ind}}$$

where $B = A^{p^t}$, $b_i = a_i{}^{p^t}$. Also from (11a)

$$A^{p^t} = \left(1 - \sum_{i=1}^{s-1} a_i\right)^{p^t}.$$

By the use of the multinomial theorem, we find that

$$\left(1 - \sum_{i=1}^{s-1} a_i\right)^{p} = \left(1 - \sum_{i=1}^{s-1} a_i{}^{p}\right) + p\gamma,$$

where γ is in $F(p^n)$ and therefore

$$\left(1 - \sum_{i=1}^{s-1} a_i\right)^{p} = 1 - \sum_{i=1}^{s-1} a_i{}^{p},$$

since if $c_1, c_1 \ldots c_{p-1}$ are the elements of $F(p)$ then $c_p = c_0$ in $F(p)$ and therefore $F(p^n)$. By proceeding in this manner we have

$$\left(1 - \sum_{i=1}^{s-1} a_i\right)^{p^t} = 1 - \sum_{i=1}^{s-1} a_i{}^{p^t},$$

and in view of our remark concerning (20) and (21) our relation (19) follows. Considering the well-known ψ for $s = 2$, investigators have found it convenient, as we did in (14), to employ numbers closely related to ψ which have many of the properties of ψ for $s = 2$. Hence we consider numbers related to the general ψ, but appear to retain properties of ψ such as (12).

Let m be the L. C. M. of $m_1, m_2 \ldots m_s$. Then $m \leqq p^n - 1$. Then we may write, if $m = t_i m_i$; $i = 1, s, \ldots s$, with $\alpha = e^{2i\pi/m}$,

$$\psi = \sum_{h_1, h_2, \ldots h_s} \alpha^{t_s \mu_s \text{ ind } H} \prod_{i=1}^{s-1} \alpha^{\mu_i t_i h_i}. \tag{22}$$

For many purposes this will turn out to be a more convenient form for ψ than either (11) or (14). Obviously also we may employ $p^n - 1$ in lieu of m, and obtain an analogous form. In connection with (22) we note also that in lieu of it we may employ the simpler form

$$\psi = \sum_{h_1,\, h_2,\, \ldots h_s} \alpha^{\mu_s' \text{ ind } H} \alpha^D, \tag{23}$$

where μ_1' and μ_s' are any integers ≥ 0, and where $\sum_{i=1}^{s-1} \alpha^{\mu'h_i} = D$. Clearly (23) includes the form (22).

We now note that in (11) we may obtain a number related to ψ by writing $(-a_k')$ in lieu of a_k in (11) letting a_k range over the same set that a_k did if k is in the set 1, 2, ...s. For this gives

$$\psi_2 = \alpha_k^{\mu_k \text{ ind } (-1)} \sum_{a_1,\, a_2,\, \ldots a_s} \alpha_s^{\mu_s \text{ ind } A'} \prod \alpha_i^{\mu_i \text{ ind } a_i}, \tag{24}$$

when we write a_k for a_k', noting that

$$\alpha^{\mu_k \text{ ind } (-a_k')} = \alpha^{\mu_k \text{ ind } (-1) - \mu_k \text{ ind } a_k}$$

and

$$A' = 1 + a_k - \sum_{\substack{i=1 \\ i \neq k}}^{s-1} a_i.$$

Now ψ_2 in (24) obviously has the same property (12). In the same way ψ_2, the number obtained by changing all the negative signs in A has the same property as well as the number ψ_1 of (14).

We note also that since p is an odd prime $g^{(p^n - 1)/2} = -1$ in $F(p^n)$, so that in (24)

$$\alpha_k^{\mu_k \text{ ind } (-1)} = \alpha_k^{\mu_k(p^n - 1)/2}.$$

We have also by definition of α_k,

$$\alpha_k^{p^n - 1} = 1, \quad \alpha_k^{(p^n - 1)/2} = \pm 1, \tag{25}$$

the sign depending on the value of m_k. In another paper[*] the writer pointed out that when α is an mth root of unity, m composite, then a relation between exponential sums found by the sole use of

$$\alpha^{m-1} + \alpha^{m-2} + \ldots + 1 = 0 \tag{26}$$

does not in general yield as much information as when we replace α by an indeterminate x in said relation and reduce the resulting polynomials modulo $(x^m - 1)/(x - 1)$. Hence we shall apply this idea to several equations given above, in order to obtain relations which are in effect more general. If we take ψ in the form given in (22) and multiply it by its conjugate imaginary, assuming the conditions (12a), we find by the use of (26) only, the relation (12), by proceeding in a manner equivalent to Weil's[8] derivation of it; now assume that we take ind H positive or zero in (22) and the μ's positive integers such that (12a) is satisfied then

$$\psi(x) \;=\; \sum_{h_1 \ldots h_s} x^{\iota_{h_1 \iota_2} \,\text{ind}\, H} \prod_{i\,-\,1}^{s\,-\,1} x^{\mu l_i h_i}$$

is a polynomial with rational integral coefficients, as is also $\psi(x^{m\,-\,1})$. Noting that $\psi(\alpha^{m\,-\,1}) = \psi(\alpha^{-1})$, we then have

$$\psi(x)\psi(x^{m\,-\,1}) \;=\; p^{(s\,-\,1)s} \;+\; C(x)\,\frac{x^m - 1}{x - 1} \tag{27}$$

where $C(x)$ is a polynomial with rational integral coefficients. Other relations such as (18) may be treated similarly.

* Most of the material in this paper was discussed in a mathematical seminar at the University of Texas and I am indebted to O. B. Faircloth, C. A. Nicol and Milo Weaver for corrections and suggestions.

[1] *Abhandlungen der Akademie su Berlin* 1770, 1771. (Cf. our footnote 5 also.)

[2] Jacobi, C. G. J., *Gesammelte Werke* Bd. VI, 254, 274; VII, 393–400. Jacobi does not seem to have given ψ_1 in an explicit form involving α alone, he employed (13) for $s = 2$ to obtain properties of ψ_1.

[2a] Oeuvres, (1), III, 112.

[3] *Trans. Am. Math. Soc.*, **17**, 167 (1916).

[4] *J. für die Math.* (Crelle) 121–36 (1852).

[5] *J. für die Math.* (Crelle), **172**, 153 (1935). They termed this number a Generalized Gauss sum. It is a direct generalization of our (1). The reference given in our first footnote to the number defined in our relation (1) is given by P. Bachmann (*die Lehre von der Kreisteilung*, Tuebner Leipzig und Berlin, 1921, p. 76) and also H. Weber (*Algebra*, vol. 1, French edition, Gauthier Villars, Paris, 1898, p. 623) the latter remarking, concerning the numbers in our (1) "connues sous le nom de resolvantes de Lagrange." Further, Hilbert (*Gesammelte Abhandlungen* Bd. 1, 225) calls our (1) a "Lagrangesche Wurzelzahl" and the "cyclotomic periods" associated with (1) a "Lagrangesche Normal-basis." The term "Lagrange resolvent" is also used in *Report on the Theory of Algebraic Numbers*, National Research Council of the National Academy of Sciences, Washington, vol. 1, p. 50 (1923). In view of these circumstances we prefer the term "Generalized Lagrange Resolvent" for (4), at least until it appears that the references given by Bachmann and Weber are incorrect. So far we have been unable to check the original sources.

[6] *Math. Ann.*, **37**, 321 (1890).

[7] These PROCEEDINGS, **35**, 97 (1949), relations (10) and (11). The relation (8) of this paper is another result from the collaboration of Dr. Hua and myself.

[8] *Bull. Am. Math. Soc.*, **55**, 500 (1949).

[9] These PROCEEDINGS, **35**, 686–90 (1949).

ON DISTORTION AT THE BOUNDARY OF A CONFORMAL MAP

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The object of the present note is to indicate the usefulness in the study of distortion at the boundary, of Carathéodory's theory of the conformal mapping of variable regions. The emphasis here is on method, for the results are in part known.[1]   Our main result is

THEOREM 1.   *Let R_w be a Jordan region of the w-plane not containing $w = \infty$ but containing the line segment $0 \leq w < 1$, and whose boundary possesses at the point $w = 1$ forward and backward tangents making equal angles $\alpha/2$ (>0) with the negative direction of the axis of reals.   Let the function $w = f(z)$ map the region $R_z : x < 1$ of the $z(= x + iy)$-plane onto R_w in such a way that we have $f(0) = 0, f(1) = 1$.   Let the sequence $x_n, 0 < x_n < 1$, approach unity. Then for z on any closed bounded set in R_z we have uniformly*

$$\lim_{n \to \infty} \frac{f[(1 - x_n)z + x_n] - f(x_n)}{1 - f(x_n)} = 1 - (1 - z)^{\alpha/\pi}, \qquad (1)$$

$$\lim_{n \to \infty} \frac{1 - f[(1 - x_n)z + x_n]}{[1 - f(x_n)](1 - z)^{\alpha/\pi}} = 1. \qquad (2)$$

The tangent line is merely the limit of the secant line through $w = 1$ as the second intersection with the curve approaches $w = 1$.

We first establish Theorem 1 for the case that R_w is symmetric in the axis of reals; here the cut $0 \leq w \leq 1$ is the image of the cut $0 \leq z \leq 1$.   The function $z' = (1 - x_n)z + x_n$ maps R_z onto itself, so the function

$$w = f_n(z) \equiv \frac{f[(1 - x_n)z + x_n] - f(x_n)}{1 - f(x_n)} \qquad (3)$$

maps the region R_z in such manner that we have $f_n(0) = 0, f_n'(0) > 0$. $f_n(1) = 1$, onto the region R_w^n in the w-plane found from R_w by stretching from the point $w = 1$ in the ratio $[1 - f(x_n)]:1$.   The kernel of the sequence of regions R_w^n is a region R_w^0, namely an infinite sector of angle α with vertex $w = 1$ and bisected by the axis of reals.   The function $w = f_0(z)$ which maps R_z onto R_w^0 with $f_0(0) = 0, f_0'(0) > 0$, is the second member of (1), and equation (1) now follows by Carathéodory's theory of the mapping of variable regions.   Equation (2) is an immediate consequence of (1).

If the points $\zeta_n = \zeta_n' + i\zeta_n''$ of R_z lie in an infinite sector S with vertex $z = 1$, of angle less than π and symmetric in the axis of reals, we set

$$x_n = \zeta_n', \qquad z_n' = i\zeta_n''/(1 - x_n), \qquad (4)$$

so the points s_n' are collinear with $s = 1$ and ζ_n, and lie in S on the axis of imaginaries, hence lie on a closed bounded subset of R_s. Equation (2) with $s = s_n'$ and $\zeta_n \to 1$ thus implies

$$\lim_{n \to \infty} \frac{1 - f(\zeta_n)}{[1 - f(x_n)][1 - s_n']^{\alpha/\pi}} = 1. \tag{5}$$

In particular if the angle $arg(1 - \zeta_n)$ approaches a limit γ, so does $arg(1 - s_n')$, and from (5) we have

$$\lim_{n \to \infty} arg[1 - f(\zeta_n)] = \alpha\gamma/\pi. \tag{6}$$

Conversely, (6) implies $arg(1 - \zeta_n) \to \gamma$. These equations merely express the well-known fact (Lindelöf) that cuts with tangents at $s = 1$ and $w = 1$ are transformed into cuts with tangents at $w = 1$ and $s = 1$, and angles at those points are transformed proportionally.

We now differentiate (1) with respect to s and again make the substitution (4); we have

$$\lim_{n \to \infty} \frac{f'(\zeta_n)(1 - x_n)}{[1 - f(x_n)](1 - s_n')^{-1 + \alpha/\pi}} = \alpha/\pi. \tag{7}$$

Division of (7) by (5) member by member, with use of the equation $(1 - x_n)(1 - s_n') = 1 - \zeta_n$ now yields

$$\lim_{n \to \infty} \frac{f'(\zeta_n)(1 - \zeta_n)}{1 - f(\zeta_n)} = \alpha/\pi, \tag{8}$$

an important relation due to Visser. It is to be noted that if $arg(1 - \zeta_n)$ approaches the limit γ, then it follows from (6) and (8) that we have

$$\lim_{n \to \infty} arg[f'(\zeta_n)] = \gamma(\alpha - \pi)/\pi. \tag{9}$$

Equation (6) implies $arg(1 - \zeta_n) \to \gamma$ and hence (9).

Theorem 1 with various corollaries is now established for the case that R_w is symmetric in the axis of reals. The proof is not valid without that assumption, for we have essentially used the fact that $f_n'(0) = f'(x_n)$ $(1 - x_n)/[1 - f(x_n)]$ is positive, or at least approaches a positive limit.

It is a consequence of (6) and (8) that the particular map $M_1: w = f(s)$ of R_w has *property A*, namely that angles at $s = 1$ or $w = 1$ bounded by curves in the given regions not tangent to the boundary but with continuously turning tangents are transformed proportionally, and the transformed angles are also bounded by curves with continuously turning tangents.[2] It follows that *any* map M_2 of R_w onto a half-plane so that $w = 1$ is invariant has property A, for the map $M_1(M_2^{-1})$ is a map of a half-plane onto a half-plane involving at $w = 1$ equality of angles bounded by curves not tangent to the boundary, hence by Schwarz's principle of symmetry this

map is conformal. Any map of R_w onto an infinite sector with vertex $w = 1$ and with $w = 1$ invariant can be accomplished by mapping R_w onto a half-plane with $w = 1$ invariant, followed by a transformation $w' = 1 - \mu(1 - w)^\beta$, $\beta > 0$, hence also possesses property A.

We have now proved, for a symmetric angle,

THEOREM 2. *On a Jordan curve C let M be an angle with vertex V at which C possesses forward and backward tangents. When the interior of C is mapped one-to-one and conformally onto the interior of a Jordan curve C' so that the sides of M in the neighborhood of V correspond to line segments, the map possesses property A, namely that any Jordan arc interior to C except for an end-point at V but with continuously turning tangent and not tangent to C is transformed into a Jordan arc interior to C' except for an end-point at the image of V but with continuously turning tangent and not tangent to C', and conversely. Angles at V are transformed proportionally.*

Let R_w in Theorem 1 no longer be symmetrical; we show that any map of R_w onto an infinite sector possesses property A. We assume, as we may do with no loss of generality, that R_w lies in an infinite sector with vertex $w = 1$ and of angle less than π. If R_w is bounded in part by a line segment terminating in $w = 1$, we may assume that no point of that line is in R_w, and we choose that segment on $-\infty < w \leqq 1$, reflect R_w in the axis of reals, and map the new region consisting of R_w plus its reflection onto a half-plane; this map transforms R_w into a quadrant and has property A. If R_w is not bounded in part by a line segment terminating in $w = 1$, we draw a line segment to the point $w = 1$ which is a cut for the exterior of R_w. A Jordan region R_w' bounded in part by this cut and in part by a side of the given angle contains R_w; any map of R_w' onto an infinite sector with vertex $w = 1$ and with $w = 1$ invariant possesses property A, and carries R_w into a region bounded in part by a line segment terminating in $w = 1$. Any further map of the latter region onto an infinite sector with vertex $w = 1$ and with $w = 1$ invariant possesses property A. Theorem 2 is completely established. Theorem 2 is due to Visser; the method of using angles bounded in part by straight lines is due in somewhat different form to Carathéodory.

We are now in a position to complete the proof of Theorem 1, where R_w is no longer symmetric. We choose an arbitrary sequence $x_n \to 1$, $0 < x_n < 1$, as before, and define the region R_w^n as the image of R_s under the transformation

$$w = f_n(s) = \frac{f[(1 - x_n)s + x_n] - f(x_n)}{|1 - f(x_n)|} \cdot \frac{|f'(x_n)|}{f'(x_n)}, \tag{10}$$

whence $f_n(0) = 0$, $f_n'(0) > 0$. The segment $0 \leqq s \leqq 1$ is a cut in R_s, and from Theorem 2 we have $\lim_{n \to \infty} f_n(1) = 1$. The kernel of the regions R_w^n is again R_w^c, so we conclude under the present hypothesis equations (1), (2),

and with the notation (4) conclude also (5), (6), (7), (8), (9). We have not merely established Theorem 1 but likewise

COROLLARY 1. *Under the conditions of Theorem 1, let S be a closed infinite sector less than π with vertex $z = 1$, S except for its vertex lying in R_z. If the sequence ζ_n lies in S and $\zeta_n \to 1$, then we have (8). In the notation (4) we have (5) and (7); if arg $(1 - \zeta_n) \to \gamma$, we have (6) and (9).*

We remark that this proof makes use of well-known results on the topological character of a conformal map, especially the images of cuts in a region, but assumes no previous results on transformation of angles on the boundary. Even in our use of maps of variable regions we do not need the classical Verzerrungssatz, for the point $w = 1$ is accessible from the exterior of R_w, and for n sufficiently large all the regions R_w^n leave uncovered a Jordan region near $w = 1$ exterior to R_w; under a suitable linear transformation the set of functions $f_n(z)$ becomes a bounded set.

Results on the higher derivatives of $f(z)$ follow readily by differentiation of equations (1) and (2).

Our proof of Theorems 1 and 2 and Corollary 1 does not essentially depend on an assumption that R_w is a *Jordan* region. It is sufficient if the boundary of R_w possesses forward and backward tangents, in the sense that there exist two half-lines terminating in $w = 1$, and given two arbitrary closed sectors with vertex $w = 1$ containing those half-lines in their interiors, there exists a neighborhood of $w = 1$ in which all boundary points of R_w lie in those sectors; it is naturally assumed that this property is not possessed by a single half-line terminating in $w = 1$ and an arbitrary closed sector containing it. As another example, in which the boundary of R_w does not possess forward and backward tangents at $w = 1$, let R_w consist of the half-plane $u < 1$ (where $w = u + iv$) plus an infinite set of canals in $u \geq 1$. These canals are to be non-overlapping, are to abut on the line $u = 1$ in segments whose mid-points are $v = v_n (\to 0)$, where the lengths of the segments are, respectively, $1/v_n^2$. Of course the canals need not be bounded by Jordan arcs, nor need R_w be symmetric in the axis of reals. It will be noted that the transformation $w' = 1 - (1 - w)^{1/2}$ transforms R_w onto a region of the w'-plane which leaves a quadrant with vertex $w' = 1$ uncovered, and the point $w' = 1$ is accessible from the exterior. Of course R_w may also be modified by the subtraction of suitable subregions of $u < 1$ adjacent to the boundary $u = 1$.

The purpose of the present note is to indicate a method (namely the use of Carathéodory's theory of variable regions) rather than to emphasize the extensive applications of the method, which are reserved for another occasion. For instance if R_w is a suitably chosen region with zero angle at $w = 1$, properly determined similarity transformations of R_w define regions R_w^n whose kernel is an infinite strip bounded by two parallel lines. Again, for certain regions R_w the kernel of a sequence of regions R_w^n defined by (3)

depends on the geometric nature of R_w and varies with the choice of the points x_n or $f(x_n)$; for instance R_w may consist of the interiors of infinitely many circles approaching the point $w = 1$ and joined by canals; information regarding the transformation of R_w can still be obtained by the present method.

[1] A recent summary of results on distortion at the boundary, with detailed references to the literature, is given by Gattegno, C., and Ostrowski, A., Mémorial des sciences mathématiques, fascicules 109 and 110 (1949).

[2] Of course property A implies that angles in the given region of either plane (assumed merely to exist as angles between tangents) are transformed proportionally into angles in the other plane.

GALACTIC AND EXTRAGALACTIC STUDIES, XX. ON THE DISTRIBUTION OF 78,000 OF THE BRIGHTER NORTHERN GALAXIES

By Harlow Shapley

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In several earlier papers in this series the distribution and brightness of galaxies with magnitudes between 13 and 18 have been reported. Because of the relation of magnitude to distance, these surveys have involved space distribution as well as surface arrangement.

In the present communication the surface distribution alone is given for nearly 80,000 objects in the northern galactic hemisphere, north of declination +41°. Since only a small fraction of the objects marked on the photographic plates is fainter than magnitude 18, the extragalactic systems here reported can be referred to as the brighter galaxies. The distribution fainter than magnitude 18, and more distant for the average galaxy than 140,000,000 light years, will be a project for several of the newer telescopes that are now or soon will be undertaking explorations deep into the metagalaxy.

1. Scope of the Survey.—The 153 plates on which this survey depends were made with the Metcalf refractor at the Oak Ridge Station by Henry A. Sawyer, using Cramer Hi-Speed Special (blue) plates all with exposures of three hours. Miss Rebecca Jones examined most of the plates and tabulated the nebular counts; Mrs. Nail has assisted in the numerical discussions.

Since the main object of this prolonged survey of northern galaxies is, first, to examine the true clustering and intrinsic distributional irregularities (as distinguished from the uneven blocking in low latitudes by interstellar obscuring clouds), and, secondly, to evaluate the relation of galactic latitude to the frequency of seventeenth magnitude systems, no fields nearer the Milky Way than galactic latitude +20° are included. This limitation has also the advantage of minimizing the misidentifications near the plate

limit that occur in the richer star fields of low latitude. It is believed that the survey is reasonably accurate, and that the number of faint double or multiple stars erroneously included (estimated at about three per. cent of the entries) is balanced, on the average, by the over-looked galaxies, which are generally of the spheroidal type. The degree of accuracy of the identifications has been sample-checked with the aid of the large reflectors.

The plates show stars to the eighteenth magnitude (median working limit is 17.9), and the galaxies are therefore recorded with essential completeness to magnitude 17.5. All galaxies within a hundred million light years, with absolute magnitudes brighter than −15, are thus included, but the dwarfs at that distance are too faint for inclusion.

The region of the sky covered includes the whole of the constellations Draco and Ursa Minor, most of Lynx and Ursa Major and considerable portions of Canes Venatici, Boötes, Hercules and Camelopardalis. It may be of interest that 1500 galaxies are shown on these Harvard plates in the bowl of the Big Dipper. If the radial density is approximately constant, there should be in this area, bounded by stars of the Dipper's bowl, more than one million galaxies photographable with long exposures on fast plates with the Hale reflector on Palomar.

2. *Summary of Observations.*—In table 1 the observational material is . summarized, with plate centers given in both equatorial and galactic coordinates. The magnitude limits, m_s, refer to stars. The galaxy census is shown in five columns. N_T refers to the whole plate (about 35 square degrees); N_{25} to the central area of 25 square degrees, which encompasses for each plate practically all that can be used without application of distance corrections to the magnitudes and to the counted numbers of galaxies. N_9 gives the total population for the central 9 square degrees, and the last two columns the data per square degree reduced to a common magnitude limit of 17.9.

3. *The Magnitude Limits.*—The equatorial and galactic coordinates in table 1 give the center of the plates within a tenth of a degree. After the plates were marked for galaxies and the magnitude limit for stars determined, the galaxies were counted with the aid of a superposed glass reticule with squares one degree on the side. There seems to be no point in recording the positions of these numerous and generally featureless extragalactic objects more closely than is here provided. The actual numbers in each square degree for each plate will be published in the *Harvard Annals* and thus permit a detailed analysis of the distribution.

The varying sky conditions and emulsion speeds have produced the differing plate limits (sixth column). The limits have been determined for each plate through the star-count method, which is based on fundamental Mount Wilson magnitudes in Selected Areas. In this northern part of the sky, away from the congestions and irregularities of the Milky Way, star

TABLE 1

POSITIONS AND COUNTS

PLATE	α (1900)		δ		λ	β	m_p	N_T	N_{28}	N_s	REDUCED $\bar{N}_{28}$	$\bar{N}_s$
27801*	0ʰ	5ᵐ1	+87°	19′	90°	+25°	17.6	112	94	25	5.7	4.2
28001*	2	2.0	83	05	93	22	18.0	141	120	34	4.2	3.3
28034*	4	7.6	83	06	96	24	17.8	105	79	25	3.7	3.2
28048*	4	18.4	77	24	101	20	18.2	173	141	36	3.7	2.6
29142	5	42.3	68	25	112	21	18.0	181	152	45	5.3	4.2
29054*	5	53.8	78	00	103	25	17.9	308	242	65	9.7	7.2
27640‡	5	55.2	72	37	109	23	18.1	378	260	74	7.9	6.2
28049*	6	23.9	82	11	99	27	18.0	411	333	98	11.6	9.5
29487	6	27.2	52	56	130	20	18.5	255	215	104	3.8	5.1
29810	6	27.6	58	11	125	22	18.3	161	126	47	2.9	3.0
29264	6	34.8	63	03	120	24	18.1	490	370	126	11.2	10.6
29216	6	40.7	67	41	115	26	18.2	523	388	111	10.2	8.1
29931	6	53.5	48	17	136	23	18.2	784	646	209	17.0	15.3
28069	6	55.6	87	11	93	28	17.7	136	123	54	6.5	7.9
29781	6	57.6	52	53	131	24	18.0	387	297	77	10.3	7.5
28733†	7	5.0	44	00	141	23	18.3	570	468	170	10.7	10.9
27521*	7	7.8	72	34	110	29	18.2	491	392	137	10.4	10.0
29213	7	8.9	58	13	126	27	18.2	619	506	140	13.3	10.3
29853	7	22.9	62	41	121	30	18.1	584	427	154	13.0	13.0
28666*	7	24.7	77	02	105	30	18.3	431	345	93	7.9	5.9
29944	7	32.2	67	46	115	31	18.1	316	261	80	7.9	6.2
29310	7	33.8	48	23	138	29	18.0	662	478	116	16.6	11.2
28753†	7	36.8	44	01	143	29	18.2	571	453	153	11.9	11.7
30052	7	46.4	52	59	133	32	18 4	944	722	306	14.4	17.0
29306	7	57.0	58	03	127	33	17.7	697	610	274	32.2	40.1
28603	8	6.3	48	35	138	35	17.9	593	478	191	19.1	21.2
29948	8	11.0	62	47	121	35	18.0	887	672	267	23.4	25.8
29493†	8	15.0	43	39	145	35	18.0	711	583	185	20.3	17.9
26003‡	8	21.2	72	38	109	34	18.5	605	506	204	8.8	9.9
30037	8	21.7	53	16	133	36	17.9	726	593	217	23.7	24.1
28261*	8	28.7	82	35	98	31	18.1	234	183	67	5.5	5.6
31364	8	38.1	58	29	126	39	17.8	369	319	108	14.7	13.8
29215†	8	38.5	44	01	144	40	18 2	926	716	214	18.9	15.7
28686	8	39.3	67	47	114	37	17.7	650	527	182	27.8	26.7
30040	8	44.6	48	54	138	41	18.1	1342	1012	394	30.8	33.3
30651	9	0.2	53	21	132	43	17.8	703	587	206	27.0	26.3
28699	9	2.5	62	49	119	41	17.8	363	292	116	13.4	14.8
28667*	9	5.7	77	28	102	35	18.2	540	448	160	11.8	11.7
27687†	9	17.9	43	23	145	47	18.4	835	663	198	13.2	11.0
29257	9	22.7	58	33	124	45	17.8	354	284	110	13.1	14.0
29877	9	24.2	48	15	138	47	17.9	1135	849	324	34.0	36.0
31367	9	31.6	67	38	112	41	17.6	406	314	130	18.9	21.7
28239*	9	33.9	72	42	106	39	18.1	482	385	128	11.7	10.8
31220	9	44.8	53	37	129	49	17.7	883	660	222	34.8	32.6
30078†	9	47.6	43	47	144	52	18.5	1226	1028	380	18.0	18.4
27701	9	50.8	62	57	116	46	18.0	607	490	157	17.1	15.1

TABLE 1   (*Continued*)

PLATE	a	(1900)	δ		λ	β	m_s	N_T	N_{36}	N_9	REDUCED $\bar{N}_{36}$	$\bar{N}_9$
29932	9	58.1	49	04	134	53	18.0	985	766	232	26.6	22.4
28965	10	6.8	58	28	120	50	18.2	1064	819	295	21.6	21.6
29278	10	19.8	53	07	126	54	17.9	1361	1069	377	42.8	41.9
29265†	10	22.1	43	29	142	58	18.4	1377	1096	420	21.9	23.3
30903	10	27.6	68	00	107	45	17.7	595	507	159	26.8	23.4
30740	10	31.2	48	47	131	58	18.1	2396	1792	612	54.5	51.7
28161*	10	34.8	77	57	98	38	17.9	518	453	164	18.1	18.2
28735	10	36.9	62	42	111	50	17.6	404	322	127	19.5	21.3
28056*	10	38.3	72	49	102	42	17.9	699	557	235	22.3	26.1
27584‡	10	50.2	58	00	115	54	17.9	815	650	216	26.0	24.0
29283†	10	54.8	43	27	137	64	18.5	1016	811	273	14.2	13.2
28868	10	55.8	53	02	130	58	18.3	820	641	185	14.7	11.8
28078*	11	4.0	82	17	94	35	17.9	333	264	89	10.6	9.9
31292	11	6.2	48	29	125	63	17.6	739	575	192	34.7	32.2
29524†	11	15.3	72	16	99	44	17.9	724	579	232	23.2	25.8
28788	11	25.2	43	43	129	68	18.1	769	594	216	18.1	18.2
29306	11	28.4	68	08	100	49	17.7	551	465	172	24.5	25.2
28109	11	32 1	63	15	103	53	17.7	1033	799	309	42.2	45.3
28719	11	35.0	58	32	106	57	18.1	1289	1035	319	31.5	26.9
28754	11	40.9	48·	19	115	67	17.8	1320	1072	399	49.3	50.9
27641‡	11	42.0	53	50	108	62	18.5	2273	1837	614	32 1	29.8
28057*	11	56.0	87	32	90	30	18.0	224	162	53	5.6	5.1
27639†‡	11	58.1	43	47	115	72	18.3	1277	974	371	22.4	23 7
28086*	12	0.5	77	27	93	40	17.6	314	258	90	15.6	15.1
28095*	12	11.1	73	00	93	45	17.7	568	470	161	24.8	23 6
28938	12	17.7	48	52	100	69	18.0	985	797	279	27.7	27.0
30797	12	18.3	53	45	97	64	17.8	1463	1066	366	49.0	46.8
28913	12	18.8	63	35	94	54	17.8	1085	907	346	41.7	44.2
27688	12	19.5	58	19	95	59	17.9	893	757	262	30.3	29.1
27704†	12	27.6	43	45	98	74	18.1	878	677	206	20.6	17.4
30125	12	35 2	67	57	91	50	17.8	858	681	256	31.3	32.7
31359	12	50.6	48	40	85	69	17.8	779	588	218	27 0	27.8
27635*	12	54.0	82	41	90	35	18.1	555	447	136	13.6	11.5
30851	12	55 4	53	24	85	64	17 8	1469	1207	394	55.5	50 4
30038†	13	04.1	43	42	74	74	18.0	1435	1172	447	40.8	43.2
30041	13	6.4	58	32	83	59	17.7	773	620	210	32.7	30.8
27194*	13	10.8	72	04	87	46	18.1	558	447	209	13.6	17.6
28213	13	13.0	62	58	83	55	17.6	510	395	144	23.9	24.2
27645*	13	23.6	77	25	87	40	18.0	973	831	321	28.9	1.1
29377	13	24.9	48	21	70	68	17.8	578	441	161	20.2	20.6
30742	13	26.4	67	32	83	50	17.8	808	581	187	26.7	23.9
30837	13	35.9	53	24	72	63	17.6	641	491	169	29.6	28.4
30129†	13	41.8	43	26	55	70	18.1	1079	890	329	27.1	27.8
29498	13	49.9	58	24	73	57	17.8	1154	890	283	40.9	36.1
30053	13	58.6	63	01	75	53	17.6	669	526	164	31.7	27.5
29447	14	04.2	48	40	58	64	17.9	723	569	179	22.8	19.9

TABLE 1 (*Continued*)

PLATE	α	(1900)	δ		λ	β	m_s	N_T	N_u	N_s	REDUCED $\overline{N_u}$	$\overline{N_s}$
28914	14	13.4	53	43	63	59	17.8	742	566	194	26.0	24.7
29962†	14	13.4	43	59	47	65	18.3	714	583	233	13.4	14.9
30132	14	18.4	68	12	77	47	17.6	723	551	198	33.5	33.2
28158*	14	32.1	72	55	79	43	17.7	354	298	108	15.7	15.8
29606	14	36.8	49	08	52	59	17.8	747	567	163	26.1	20.8
28947	14	38.3	58	22	64	53	17.4	561	441	158	35.2	35.2
29979†	14	42.8	43	51	41	61	18.1	639	474	142	14.4	12.0
29559	14	48.9	63	11	68	49	17.4	691	582	228	46.6	50.6
28172*	14	53.5	77	48	82	38	17.7	425	363	128	19.1	18.7
30927	14	54.3	53	44	56	55	17.5	730	560	215	39.0	41.6
27088*	15	2.7	84	20	86	33	17.8	511	411	157	18.9	20.0
28949†	15	10.5	43	25	37	56	18.0	550	415	127	14.4	12.3
30929	15	13.6	48	26	45	54	17.5	523	430	172	29.9	33.2
29504	15	16.5	58	50	60	49	17.5	401	337	133	23.5	25.7
30880	15	19.4	68	8	71	44	17.5	780	622	240	43.3	46.5
27373*	15	29.1	72	54	75	40	17.9	384	312	90	12.5	10.0
32917	15	31.2	53	21	51	50	18.0	824	672	236	23.4	22.8
32932	15	44.6	48	7	42	49	18.1	834	668	260	20.3	22.0
27406	15	45.9	62	54	63	44	18.0	649	488	182	17.0	17.6
30079†	15	48.9	44	00	36	49	18.2	1726	1425	462	37.6	33.8
27462*	15	55.4	75	05	76	37	17.8	399	281	105	12.9	13.5
28945	16	00.1	58	50	56	44	17.5	518	421	160	29.2	31.0
27192*	16	4.4	79	19	80	34	17.9	623	470	161	18.8	17.9
27273*	16	8.9	87	44	88	29	17.5	274	223	94	15.5	18.1
29556	16	13.4	53	28	49	44	17.6	597	481	171	29.0	28.7
30886	16	21.4	68	05	67	38	17.8	959	712	202	32.8	25.8
31445	16	23.3	48	28	42	43	17.6	386	321	86	19.3	14.5
30186†	16	25.3	43	34	35	43	18.0	1143	939	310	32.7	29.9
29529	16	31.6	63	04	60	39	17.3	611	475	149	43.5	37.8
27230*	16	46.3	71	55	71	35	18.2	770	659	204	17.4	15.0
28940	16	46.3	57	39	53	38	17.5	435	358	107	24.9	20.7
31418	16	51.9	53	11	47	38	17.7	602	465	185	24.6	27.2
29537†	16	54.4	43	48	35	38	17.9	500	367	109	14.7	12.1
31441	16	57.8	48	24	41	37	17.7	537	402	125	21.2	18.3
29539	17	15.5	67	29	65	34	17.5	501	366	111	25.4	21.4
28302	17	22.8	62	50	59	33	17.8	356	273	104	12.5	13.2
30123†	17	26.6	43	29	36	32	18.3	808	616	236	14.1	15.1
27320	17	29.3	57	56	53	33	17.9	481	416	144	16.6	16.0
29564	17	30.1	52	39	47	32	17.8	503	400	145	18.4	18.5
29567	17	36.6	48	29	42	31	17.6	465	370	148	22.3	24.8
27279*	17	58.0	77	22	76	30	17.8	605	504	172	23.2	22.0
30224†	17	58.1	43	32	38	28	17.7	442	373	153	19.7	22.4
27288*	18	1.6	72	24	70	29	17.8	548	440	153	20.2	19.6
27445*	18	3.4	83	26	83	29	18.2	447	355	108	9.4	7.5
28939	18	5.9	62	56	59	29	17.5	315	260	92	18.1	17.6
32320	18	9.4	48	12	43	25	17.6	153	131	54	7.9	9.1

TABLE 1 (*Continued*)

PLATE	α (1900)		δ	λ	β	m_t	N_T	N_m	N_s	REDUCED $\bar{N}_m$	$\bar{N}_s$
29618	18	10.3	67	52	65	29	17.7	332	287	90	12.5	13.2
32975	18	13.4	57	54	54	27	17.9	421	379	171	15.2	19.0
28948	18	14.0	53	15	49	26	17.7	211	160	55	8.4	8.0
28950†	18	33.9	43	08	39	20	18.0	222	189	69	6.6	6.7
32464	18	45.9	48	34	45	20	17.3	114	92	28	8.5	7.1
32469	18	49.6	52	47	50	21	17.3	208	158	51	14.4	13.0
30939	18	56.3	58	03	55	21	17.7	259	186	51	9.8	7.5
27431	19	01.7	62	32	60	22	17.8	335	280	76	12.9	9.7
28478*	19	11.4	72	04	70	24	17.8	330	266	97	13.1	12.4
28328	19	12.6	67	28	66	23	18.1	292	256	99	7.7	8.4
27293*	19	27.7	77	41	77	25	18.0	225	182	76	6.4	7.3
27753*	19	37.2	82	32	82	26	18.2	255	210	68	5.5	5.0
27796*	21	8.4	77	42	80	20	17.7	62	47	13	2.5	1.8
27806*	22	2.6	82	22	85	22	17.8	61	52	15	2.4	1.9
27837*	23	57.3	82	22	89	20	17.8	61	50	16	2.3	2.1

    * Shapley and Jones, *Harv. Ann.*, 106 No. 1 (1938).

    † Shapley and Jones, these Proceedings, 26 554–561 (1940), Harvard Reprint 208.

    ‡ Seyfert, *Harv. Ann.*, 105 No. 10 (1937).

counts provide a reliable procedure. The magnitude limits for the 153 plates are distributed as follows:

Magnitude limit	17.3	17.4	17.5	17.6	17.7	17.8	17.9
Number of fields	3	2	9	13	18	28	17
Reduction factor	2.29	2.00	1.74	1.51	1.32	1.15	0.00

Magnitude limit	18.0	18.1	18.2	18.3	18.4	18.5
Number of fields	18	17	13	7	3	5
Reduction factor	0.87	0.76	0.66	0.575	0.50	0.437

The reduction factor in the last line is based on the customary assumption, which the Harvard and Mount Wilson studies have both supported, that the average space density of galaxies in high latitudes is essentially uniform, at least out to a distance of 10^8 light years. The reduction factor has been applied only for the last two columns of table 1, and for figure 1 and table 3.

4. *Test of Metcalf Telescope Field.*—The curved glass plates so accurately fit the field of the Metcalf doublet that no appreciable distance error is found within a circle of $2^1/_2°$ radius. There may be, however, slight inequalities in the field, as is shown by the data of table 2, which are based on the nebular counts on the 153 plates. (No correction of any kind has been applied to the data in this tabulation.) In each square are the number of the square, the mean population of galaxies $\bar{N}$ for that square, the deviation $\bar{N}-20.4$, and the mean error ϵ of $\bar{N}$ (based on 153 fields). For the whole plate $\bar{N}$ averages 20.4 ± 0.2 (m. e., based on 25 squares), and ϵ averages ±0.51 ± 0.01 (m. e.).

The deviations $\bar{N}-20.4$ provide a test of the uniformity of the field of the telescope. In a sense, we are here using some 80,000 galaxies to test, through the deviations from the value 20.4 galaxies per square degree, the performance of a 4-lens objective. The test is not absolute, of course, because irregularities of nebular distribution and observational uncertainties also enter, and are not wholly smoothed out by the large number of plates. The deviations from 20.4 are for 16 of the squares less than twice the corresponding mean error ϵ, and therefore show no sensible distortion of the telescopic field at those points. Only for squares 1, 3, 14, 22, 23 and 24 does the evidence suggest that the effectiveness is definitely more or less

TABLE 2

TEST OF THE TELESCOPE'S FIELD

(See text, section 2, for description)

25	24	23	22	21
20.6	21.9	22.4	23.3	19.7
+0.2	+1.5	+2.0	+2.9	−0.7
±0.57	±0.60	±0.56	±0.60	±0.49
20	19	18	17	16
20.0	19.5	19.2	20.1	19.2
−0.4	−0.9	−1.2	−0.3	−1.2
±0.52	±0.48	±0.40	±0.44	±0.56
15	14	13	12	11
20.2	19.0	19.3	20.6	19.6
−0.2	−1.4	−1.1	+0.2	−0.8
±0.50	±0.52	±0.40	±0.48	±0.51
10	9	8	7	6
20.5	19.5	19.8	21.4	20.8
+0.1	−0.9	−0.6	+1.0	+0.4
±0.49	±0.49	±0.45	±0.49	±0.50
5	4	3	2	1
19.6	21.1	23.0	21.4	18.2
−0.8	+0.7	+2.6	+1.0	−2.2
±0.44	±0.53	±0.58	±0.55	±0.56

than the average. If rich clusters of galaxies should accidentally fall on the same square for several plates, a large positive average deviation might result. If vacancies fortuitously combine on a square, a large negative average deviation could occur. To test further this possibility of clustering, the fields of table 1 were separated into three equal groups, in order of number. The deviations are positive and large in all three groups for square 22 only; and for square 14 only, large and negative (deficiency of recorded galaxies). We conclude that the inequalities of table 2 are primarily due to clustering and fortuitous "holes," and not to instrumental causes.

The deviations of table 2 are a very sensitive measure of the telescope's field. The largest are about 12 per cent of the mean nebular numbers for

those squares. On the usual uniform-density assumption, this percentage corresponds to a deviation of only 0.08 mag. in effective reach. The average is 5 per cent, corresponding to 0.04 mag. In practice, therefore, for nebular surveys, the field inequalities are negligible. If they exist, they probably arise from differences in sharpness of focus; either the lenses or the curved-plate matrix may be at fault. With smooth fields of stars rather than patchy nebular fields, this testing method for large field lenses or mirrors could be made very precise.

5. *Distribution of Galaxies in Latitude and Longitude.*—A comparison of the results obtained from the study of the 3600 square degrees, reported in the present communication, with other areas surveyed in the southern sky, will be made in the course of a forthcoming general summary of the Harvard work on the distribution of galaxies. The summary will deal with the distribution of the brighter systems for the whole sky, and for a large fraction of the sky will concern the more distant objects down to magnitude 18, of which well over half a million have been photographed with the Harvard telescopes, but less than 200,000 studied in detail.

TABLE 3

MEAN NUMBER OF GALAXIES PER SQUARE DEGREE

GAL-ACTIC LATI-TUDE	LONGITUDE INTERVALS				
	30–59°	60–89°	90–119°	120–149°	30–149°
20–29°	12.1 (9)	9.2 (12)	7.2 (11)	11.1 (10)	9.7 (42)
30–39°	18.8 (9)	22.6 (10)	11 6 (10)	21.1 (7)	18.3 (36)
40–49°	23.0 (6)	26.0 (9)	20.7 (9)	24.5 (7)	24.5 (31)
50–59°	26.6 (5)	31.0 (7)	31.8 (7)	28.6 (7)	29.7 (26)
>60°	19.4 (4)	34.6 (5)	33.5 (6)	22.3 (8)	28.8 (18)
>40°	25.2 (15)	29.7 (21)	27.7 (22)	25.8 (17)	27.4 (75)
All	19.9 (33)	22.3 (43)	18.7 (43)	20.5 (34)	20.4 (153)

Two results from the earlier analysis of a part of the material of table 1, both of significance in the cosmography of external galaxies, are confirmed by the present larger study. The first is the demonstration of the far extension of the "Cepheus flare" or cloud of absorbing material that comes out of the Milky Way between galactic longitudes 70° and 110° and extends up to galactic latitude 37° in longitude 105°. This flare of absorption covers the North Celestial Pole, and as pointed out in earlier reports, indicates that the North Polar magnitude standard sequence is somewhat obscured. The color as well as the brightness of the standard stars must be affected. This absorbing cloud is shown in latitudes higher than +20° in the density diagram in figure 1, where each circular area is centered on a plate center and covers 20 square degrees. The actual coverage of the sky is more complete than the diagram indicates, since the plates actually cover 35 square degrees.
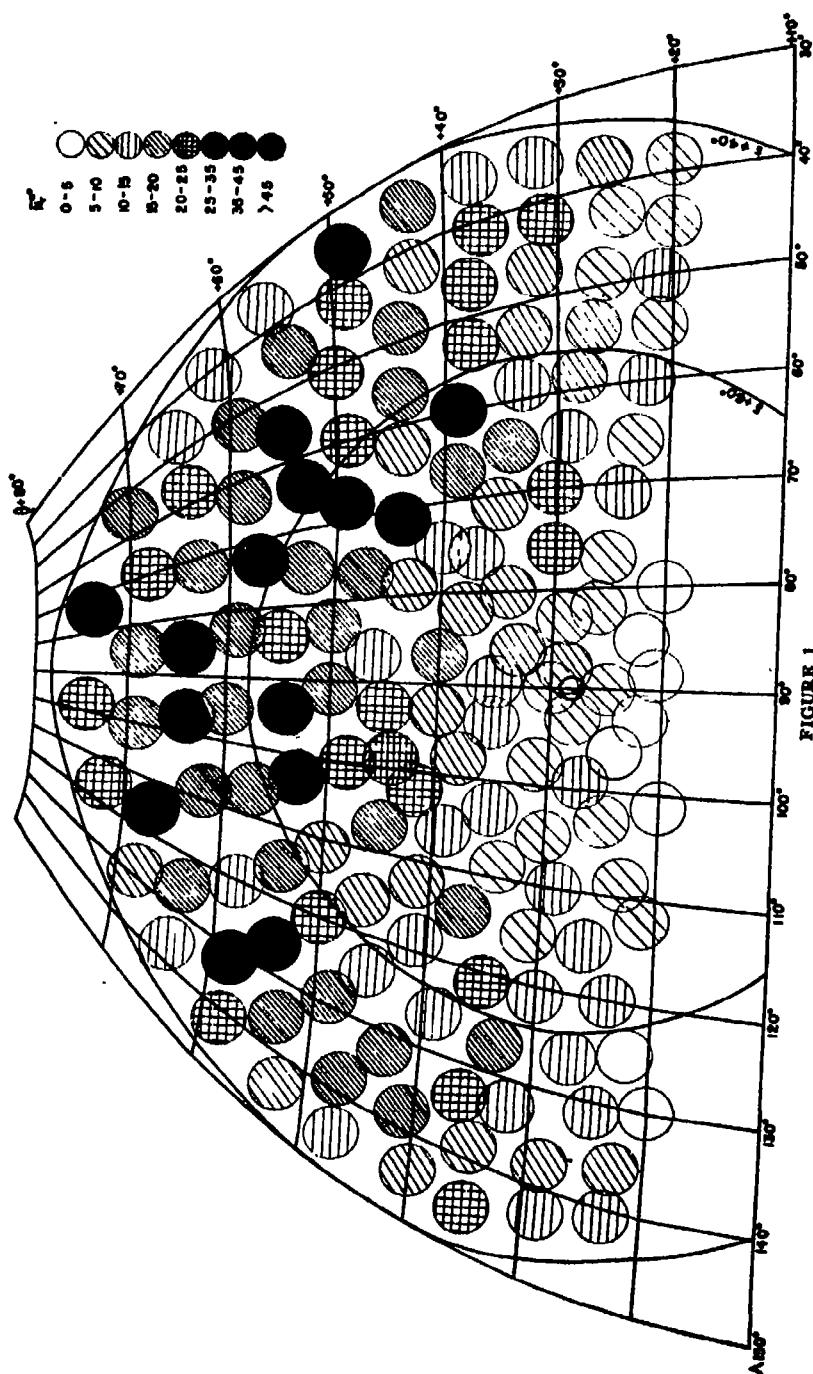
FIGURE 1

Distribution of northern galaxies.

The second earlier result supported by this survey is the evidence that from galactic latitude +40° to the North Galactic Pole there is no appreciable increase of population density with latitude. This result was first reported in the Darwin Lecture in 1934[1] and later shown for the 5° declination belt (+41° to +46°) by Shapley and Jones,[2] who used in part some of the material included in table 1. The relation of population to latitude is summarized in table 3. The mean numbers per square degree have been reduced for this tabulation to a common apparent magnitude, $m_s = 17.9$. The greatest density, when all longitudes are considered, is in the neighborhood of galactic latitude +60°.

6. *General Irregularity in Distribution.*—The clumpiness of galaxies is now generally recognized, and the density chart in figure 1 is consistent with the results we have been reporting for more than a decade. The census tables to be published later will show the irregularities in finer detail. Except for the region of the Cepheus flare, obscuration by interstellar clouds appears to be ineffective for the high latitude regions. As stated above, the mean residuals of the mean numbers for each square degree (see table 2) are a measure of the inequalities from plate to plate. The average for all squares is ±0.51, but when the fields are examined for these population irregularities at different galactic latitudes we find the following results:

Latitude interval.....	20–30°	30–40°	40–50°	50–59°	> 59°
Number of plates.....	42	36	31	22	22
Average mean residual	±0.65	±0.99	±1.16	±1.87	±1.84

The increase with latitude of the average mean residual when reduced to a common number of plates is roughly proportional to the square root of the average number of galaxies, and therefore we conclude that the irregularities in the distribution, as might be expected, are independent of galactic latitude.

A number of rich clusters or clouds of galaxies appear in the area covered and doubtless an extension of the survey to magnitude 19 and fainter will reveal many new clusters and clarify others that are here only suggested. The distribution chart given in figure 1, which is based on the reduced plate averages of table 1, show a number of very rich regions, many of which have now been further photographed with the Jewett-Schmidt telescope at Oak Ridge.

[1] Shapley, H., Harvard Reprint 105 (1934); *Mon. Not. R. A. S.*, 94, 813 (1934).

[2] These Proceedings, 26, 599–604 (1940), Harvard Reprint 209; see also Harvard Reprint, Series II, 23 (1948); *Sci. Mon.*, 67, 247 (1948).

EVIDENCE FOR THE PARTICIPATION OF KYNURENINE AS A NORMAL INTERMEDIATE IN THE BIOSYNTHESIS OF NIACIN IN NEUROSPORA*

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Investigations on the mechanism of the biological synthesis of niacin have implicated tryptophan and 3-hydroxy-anthranilic acid as niacin precursors in both Neurospora[1, 2] and the rat.[3, 4] 3-Hydroxy-anthranilic acid has been shown to serve as a normal intermediate in niacin synthesis in Neurospora,[5] and a mechanism for the conversion of this compound to niacin has been suggested.[6] It was not until recently, however, that conclusive proof was obtained that tryptophan normally serves as a major precursor of niacin in Neurospora.[7] As yet no clear proof of the identity of the intermediates between tryptophan and 3-hydroxy-anthranilic acid has been obtained. Kynurenine and 3-hydroxy-kynurenine have been proposed as intermediates in the conversion of tryptophan to niacin in Neurospora.[8] Kynurenine has been tested for its ability to support the growth of rats maintained on a niacin deficient diet and found inactive.[8] However, recent evidence presented by Heidelberger[9] and by Kallio[10] suggests that kynurenine may play a role in the conversion of tryptophan to niacin by the rat.

In the Neurospora investigations evidence substantiating the proposal that kynurenine and 3-hydroxy-kynurenine function as natural precursors of niacin has not yet been presented. Although both of these substances have been tested and found highly active in supporting the growth of certain niacinless strains,[1, 11] proof of their actual participation in niacin synthesis is still lacking. This lack of evidence is primarily due to the fact that mutant strains capable of utilizing 3-hydroxy-anthranilic acid and niacin but incapable of using tryptophan have not been studied. Mutants of this class might accumulate one or more of the intermediates between tryptophan and 3-hydroxy-anthranilic acid, and accumulation of either kynurenine or 3-hydroxy-kynurenine would constitute direct proof of the participation of these compounds in the biosynthesis of niacin in Neurospora. A mutant strain has been obtained[5] which can use 3-hydroxyanthranilic acid, cannot use tryptophan and does accumulate a kynurenine-like compound. The present paper deals with the isolation and identification of this substance and the significance of this accumulation.

Experimental.—The basal medium used throughout is the customary Neurospora minimal.[12] Cultures were grown 72 hours at 25°C. in 125-ml. Erlenmeyer flasks containing 20 or 40 ml. of medium for growth tests. At the end of the period of incubation the mycelial pads were removed,

dried and weighed.   Test samples were added to the assay flasks before autoclaving.

Table 1 lists the various Neurospora mutants used in this investigation and the substances known to support their growth.

Strain Y-31881 is biochemically distinct from the other mutant strains listed, since the group of compounds which will support its growth is characteristic of it alone.   The growth of this strain is supported by any one of the following compounds: 3-hydroxy-kynurenine,† 3-hydroxy-anthranilic acid, quinolinic acid at high concentrations and niacin.   Tryptophan, its . precursors or kynurenine cannot replace niacin for this mutant.   Genetic tests demonstrate that Y-31881 differs from the parental strain by a single gene mutation.   That this strain differs genetically from the other strains listed in table 1 was concluded from the fact that it forms a heterocaryon (as a test for allelism)[15] with these strains, and that from crosses with each of the strains listed in table 1, nutritionally wild-type progeny were recovered.

TABLE 1

GROWTH OF VARIOUS STRAINS OF NEUROSPORA

STRAIN NO.	NO ADDITION	ANTHRANILIC ACID	INDOLE	TRYPTOPHAN	KYNURENINE	α-N-ACETYL KYNURENINE	3-HYDROXY-KYNURENINE	3-HYDROXY ANTHRANILIC ACID	QUINOLINIC ACID	NIACIN
75001	−	+	+	+	+	−	?	−	−	−
10575	−	−	+	+	−	−	−	−	−	−
39401	−	−	+	+	+	+	+	+	±	+
Y-31881	−	−	−	−	−	−	+	+	±	+
4540	−	−	−	−	−	−	−	−	±	+
3416	−	−	−	−	−	−	−	−	−	+
Y-31881-3416	−	−	−	−	−	−	−	−	−	+
Wild type	+	+	+	+	+	+	+	+	+	+

On the basis of these observations it was concluded that this mutant is genetically blocked in the conversion of kynurenine to 3-hydroxy-anthranilic acid.   The double mutant Y-31881-3416 permitted a direct check on this interpretation.   Strain 3416 is a niacin-requiring mutant which cannot use 3-hydroxyanthranilic acid in place of niacin, and accumulates quinolinic acid freely in its culture medium.[6]   If the Y-31881 block interfered with the synthesis of 3-hydroxy-anthranilic acid, which in turn is known to give rise to quinolinic acid, it would be expected that this double mutant should no longer accumulate this acid.   The opposite mating types of the two strains concerned were crossed and ascospores were isolated in order from several asci.   In one ascus four wild-type spores were obtained, indicating that the other four must be double mutants.   Crossing a presumed double mutant with wild type gave both individual mutant types, verifying the genotype of the double mutant.   Y-31881-3416 and 3416,

one of the parental types, were grown on a medium supplemented with ni-
acin and the amount of quinolinic acid accumulated determined. It can
be seen from table 2 that the double mutant Y-31881-3416 does not ac-
cumulate an appreciable amount of quinolinic acid when compared to
strain 3416.   The Y-31881 block must then prevent an enzymatic reaction
which normally leads to the production of a quinolinic acid precursor.

Cross-feeding experiments were performed to test for the accumulation
of an active niacin precursor in the culture filtrates of strain Y-31881.
Strain 39401 was selected as the assay organism since this strain would be
expected to grow in the presence of indole, tryptophan or any of the inter-
mediates between tryptophan and niacin (see table 1).   The results of a
typical experiment are shown in figure 1.   It is clear that these filtrates
contain a substance or substances which support the growth of strain 39401
but not strain Y-31881.   Since the growth of strain 39401 alone is supported
by Y-31881 culture filtrate, it may be concluded that the accumulated sub-
stance is not 3-hydroxy-kynurenine, 3-hydroxy-anthranilic acid, quinolinic
acid or niacin, since both strains are equally sensitive to these compounds

To obtain sufficient material for the isolation of the active substance
(designated as 31881-I), strain Y-31881 was grown in 5-gallon bottles con-

TABLE 2

Quinolinic Acid Accumulation by Various Mutant Strains of Neurospora

STRAIN	SUPPLEMENT	γ-QUINOLINIC ACID PER CC. FILTRATE	γ-QUINOLINIC ACID PER MG. DRY WEIGHT
3416 *	20 γ-nicotinamide	50.5	8.3
Y-31881-3416	20 γ-nicotinamide	0.49	0.14

taining 18 liters of half-strength minimal medium supplemented with 4
mg. of nicotinamide.   Half-strength minimal was used instead of normal
strength since it did not decrease the amount of 31881-I accumulated yet
reduced the difficulty of isolation.   The cultures were incubated at 25°C.
under continuous aeration.   After 7–10 days of growth the mycelium was
removed by filtering through cheesecloth.   The filtrates from two bottles
were combined and concentrated *in vacuo* to approximately two liters.
The concentrate was filtered to remove insoluble material, which was then
washed twice with ethanol.   The washings were added to the clear con-
centrate.   Two volumes of ethanol were added and the mixture cooled
overnight.   The precipitate formed was filtered off and washed as before.
The clear solution was evaporated almost to dryness, brought up to a vol-
ume of 300 cc. with distilled water and acidified to pH 3.5–4.0.   It was then
continuously extracted with ether for 48 hours.   The ether extract was
concentrated to ca. 10 cc. and enough ethanol added to bring the final
volume to 60 cc.   This extract was chromatographed using whole sheets of
Whatman No. 1 filter paper cut to 17 × 20 inches.   Twelve sheets were

run at the same time and constituted a single batch. Five cc. of the ethanol extract were applied to each sheet by means of a modified kymograph previously described.[14] The sheets were then developed as ascending chromatograms with a butyl alcohol, propyl alcohol, water (1:2:1) solvent made 0.005 M with respect to ammonia immediately before use. After 24 hours the sheets were removed and air-dried.
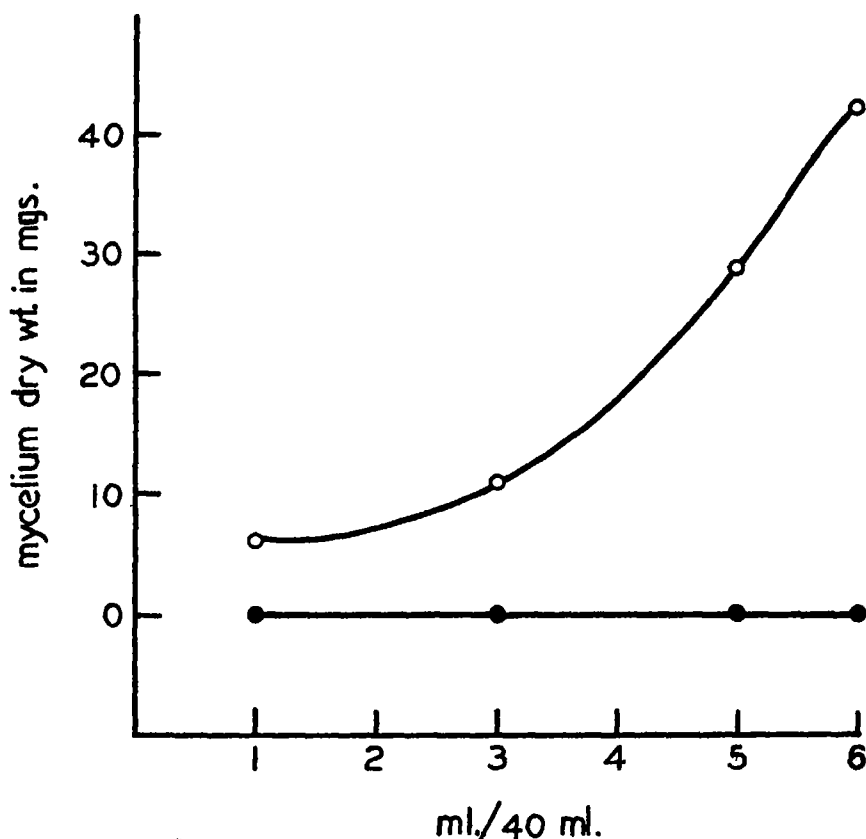
FIGURE 1

Growth of strains 39401 and Y-31881 on culture filtrates of strain Y-31881. O—O strain 39401, ●—● strain Y-31881.

The band of active material was next located in the following manner. Sterile modified Neurospora minimal medium was inoculated with a filtered conidial suspension of the test strain and poured into large (5$^{1}/_{2}$ × 16 inch) plates.[15] The medium used contained inorganic salts, 2% agar, 0.25% sucrose and 1% sorbose, this latter sugar being used to prevent spreading growth of the mycelium.[16] Strains 39401 and Y-31881 were

used as test organisms.   Two vertical sections each 1 cm. in width were
cut from one sheet of each batch.   These strips were sterilized by exposure
to a Sterilamp for ca. 15 minutes, after which one strip was placed on a
39401 plate and its duplicate on a Y-31881 plate.   Each plate could ac-
commodate 3 strips with sufficient space between them to prevent over-
lapping of the growth zones.   The plates were then maintained at 25°C.
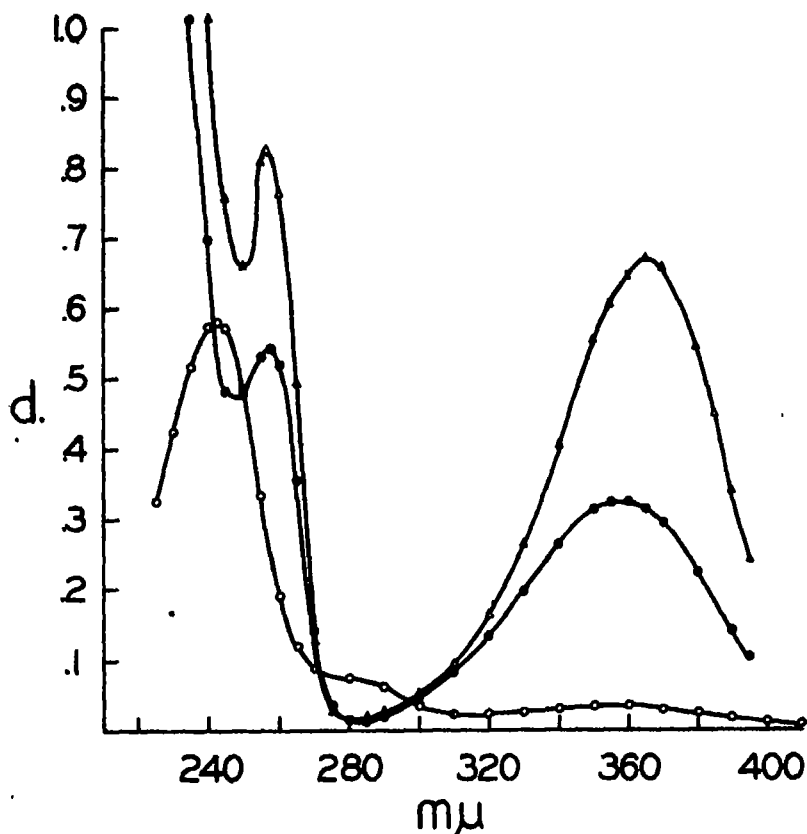
FIGURE 2

Absorption spectra of 31881-I in various solvents.   O–O 0.1 *N* HCl;   ●–● 0.1 *N* NaOH;
△–△ abs. ethanol.

for 24 hours.   Contaminants were seldom encountered because of the
short period of incubation.   The areas supporting the growth of 39401
but not Y-31881 were marked and recorded, and the corresponding sec-
tions from all sheets of the same batch were cut out and combined.   These
sections were eluted with dilute ammonia in a Waring blendor.   The
suspension was filtered to remove the paper pulp, this process repeated

and the eluates combined. The paper pulp was tested for residual activity before being discarded, and it was seldom found necessary to elute a third time. The combined eluates were evaporated to dryness *in vacuo* and the minimum amount of hot ethanol added to effect complete solution. On cooling, light brown needles which supported the growth of strain 39401 appeared. The needles were filtered off and recrystallized from water after norite treatment. This material remained active for growth of strain 39401, but was found to be only about one seven hundredth as active as niacin. After the original isolation procedure was evolved and several mg. of 31881-I were obtained, it was found that the isolation procedure could be considerably shortened. The ether extract was dissolved in hot ethanol, and when the solution had been kept in the refrigerator for a few days, crude crystals of 31881-I appeared. These crystals were filtered off and recrystallized as before.

The product in either case is pale yellow, melts from 190–195°C. (uncorr.) and decomposes at 210°C. with the sublimation of a second substance. The sublimate melts at 237–240°C. (uncorr.) when heated rapidly. The absorption spectra of the isolated material (31881-I) in several solvents are given in figure 2. Of the many substances tested only *o*-aminoacetophenone showed approximately the same absorption spectra in the solvents used, suggesting a possible relationship.

The isolated material is soluble in methanol, ethanol and acetic acid, slightly soluble in water and very slightly soluble in ether. Equivalent weight determinations gave a value of 250. The fact that molecular weight determination by ebulliometry gave values around 250, suggests that the equivalent weight determined is the molecular weight of the substance. Tests for functional groups indicated the presence of an aromatic amino group, a free carboxyl group and the absence of free α-amino, phenolic and alkoxyl groups. When 31881-I was subjected to acid hydrolysis (1 N H_2SO_4 at 100°C. for 2 hours), a considerable increase in niacin activity was noticed (Fig. 3). It was also found that the hydrolyzed solution was active for strain 75001 (see table 1) and now gave a positive test for an α-amino group. These facts suggested that the compound formed on hydrolysis was kynurenine. To establish this fact the isolation of kynurenine was attempted. One hundred mg. of 31881-I were hydrolyzed with 1 N H_2SO_4, the H_2SO_4 content brought up to 5% (by volume) and enough ethanol added to make an 80% solution. After two days in the refrigerator the colorless crystals were filtered off. These needles gave a strong qualitative test for kynurenine, contained sulfate, were active for 75001 and 39401 and appeared in all tests to be identical with kynurenine sulfate. It was also necessary to determine the nature of the group removed by acid hydrolysis in order to establish the structure of 31881-I. Since the α-amino group was freed by acid hydrolysis it was suspected that the mask-

ing group was either a formyl or an acetyl group.   On acid hydrolysis these groups would give rise to formic and acetic acids.   A 100-mg. sample of 31881-I (in 10 cc. of 1 N H_2SO_4) was hydrolyzed in a sealed glass tube placed in a boiling water bath for $1^1/_2$ hours.   The tube was removed, cooled, opened and the contents poured into a distilling flask.   The volatile acids were distilled over and the Duclaux distillation constants determined.   The constants agreed perfectly with those found using a known
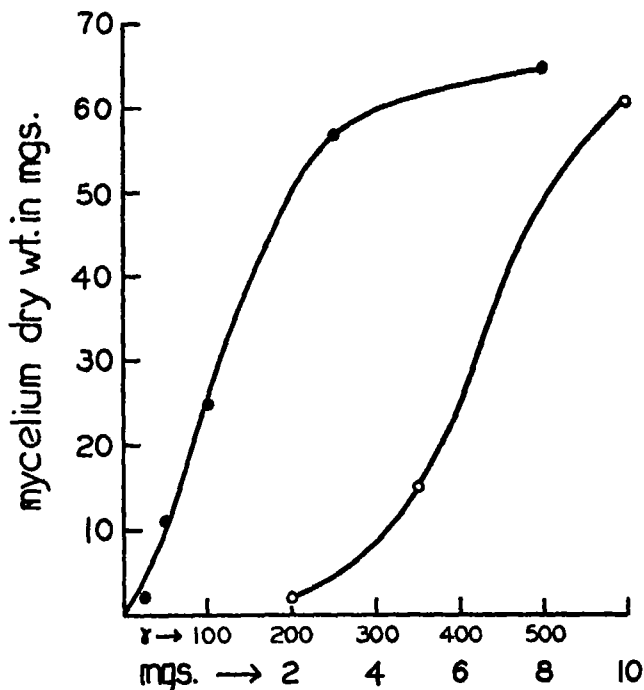
FIGURE 3

Activity of 31881-I for strain 39401 before and after acid hydrolysis.   O—O before acid hydrolysis—mg. scale;   ●—● after acid hydrolysis—γ scale.

acetic acid solution.   Furthermore, 0.36 meq. of acid was present in the 100 cc. of distillate.   This amount of acetic acid accounts for 90% of the theoretical amount of acid which would be liberated by the complete hydrolysis of 100 mg. of α-N-acetyl kynurenine.   C-H analysis of 31881-I is compared below with that calculated for α-N-acetyl kynurenine.

	c	H
Found	57.69	5.77
Calculated for $C_{13}H_{14}O_4N_2$	57.58	5.63

On the basis of these data we have identified 31881-I as α-N-acetyl kynurenine (formula I). Final proof awaits synthesis of this compound, which is now in progress.
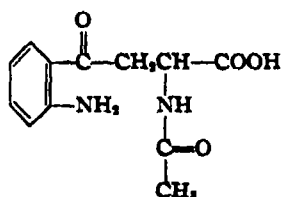
Formula I

To arrive at any conclusions concerning the significance of the accumulation of 31881-I it was necessary to determine whether other strains accumulate this substance. Several years ago Bonner and Beadle[17] reported the isolation of a substance (designated as 4540-II) from the filtrates of strain 4540. Because only small quantities of 4540-II were present and its activity slight, attention was focused on the more active substance accumulated by this strain, 3-hydroxy-anthranilic acid. From the data presented in their paper it can be concluded that 4540-II and 31881-I are identical. The C-H analyses of 4540-II and 31881-I are compared below:

	C	H
4540-II	57.55	5.68
31881-I	57.69	5.77
Calculated for $C_{11}H_{14}O_4N_2$	57.58	5 63

Filtrates from other strains were also tested for the presence of this compound. In these experiments the standard isolation and detection procedures were employed which are reported in this paper. The strains tested were 2198 and 5256 (wild type). 31881-I could not be detected in the filtrates of either of these strains. However, this method could not be expected to detect small quantities of this substance which may be normally formed.

Discussion.—Tryptophan and kynurenine have been proposed as intermediates in the synthesis of niacin in Neurospora largely because they support the growth of one genetic type of niacin-requiring mutant strain.[1] 3-Hydroxy-anthranilic acid similarly shows niacin activity[2] but proof of its natural participation in niacin synthesis has been obtained.[5] On the basis of these observations a scheme was proposed involving tryptophan, kynurenine, 3-hydroxy-kynurenine and 3-hydroxy-anthranilic acid as precursors of niacin. Certain observations regarding the strain upon which the participation of tryptophan and kynurenine have been predicated are not readily reconcilable with the thesis that tryptophan and kynurenine serve as major niacin precursors.[5,18] However, N[15] experiments, designed specifically to test this point, have shown conclusively

that tryptophan is the main source of niacin in Neurospora.[7] Also consistent with the proposed scheme is the fact that 3-hydroxy-kynurenine has been found active in replacing niacin.[11] However, final proof for the participation of kynurenine and 3-hydroxy-kynurenine depends upon the demonstration that these compounds can be and are synthesized by Neurospora. The work reported in this paper has shown that a metabolite of kynurenine is abnormally accumulated as a result of a genetic block in one strain. Therefore, proof has been obtained for the ability of the mold Neurospora to produce kynurenine-like compounds. Furthermore, this accumulation is associated with the inability of the strain concerned to synthesize niacin. There is a direct relationship between the two. Consistent with these observations is the fact that this mutant will grow in the presence of 3-hydroxy-kynurenine but not in the presence of kynurenine. Hence, it apparently cannot convert the latter substance to the former. Thus, it has been shown that Neurospora can and does synthesize kynurenine-like compounds and that these compounds are involved in niacin synthesis. In rats, the normal excretory product of kynurenine is either kynurenic or xanthurenic acid, depending upon the B_6 content of the diet. In view of the small quantities of α-N-acetyl kynurenine formed by strain 4540,and the larger quantities formed by strain Y-31881, it appears that this compound represents a major end-product of kynurenine metabolism in Neurospora. However, both kynurenic and xanthurenic acids might reasonably be expected to be found as kynurenine metabolites in Neurospora under certain conditions.

The following scheme represents the present status of the investigations on niacin synthesis in Neurospora. The vertical arrows indicate compounds accumulated as a result of the indicated genetic block.

```
                           Y-31881
tryptophan ─────→ kynurenine ──/──→ 3-hydroxy- ─────→ 3-hydroxy-anthranilic acid
                       ↓              kynurenine                    ↓
                  α-N-acetyl kynurenine                        accumulated

       4540                    3416
       ──/──→ intermediate ──/──→ niacin
                  ↓
            quinolinic acid
```
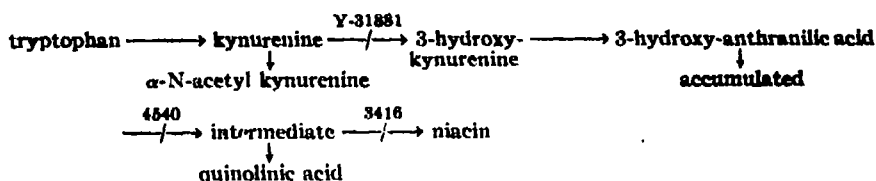
Summary.—The isolation and identification of a substance possessing slight niacin activity for one mutant strain of Neurospora has been described. The strain accumulating this substance can synthesize niacin from 3-hydroxy-kynurenine or 3-hydroxy-anthranilic acid but is incapable of utilizing tryptophan or kynurenine for this purpose. If the proposed pathway from tryptophan to niacin in Neurospora is correct the accumulated compound should be kynurenine or some product of kynurenine metabolism. Such a product has been isolated and identified as α-N-acetyl kynurenine. The accumulation of this kynurenine-like compound as a result of an induced genetic block is considered direct evidence for the participation of kynurenine in the biosynthesis of niacin in Neurospora.

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† The authors wish to thank Dr. W. Weidel of the Kaiser-Wilhelm Institut für Biochemie, who generously made a sample of 3-hydroxykynurenine available to us.

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THE EFFECT OF OXYGEN CONCENTRATION ON THE RATE OF X-RAY INDUCED MUTATIONS IN DROSOPHILA

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Various supplemental agents have been administered to organisms being exposed to x-rays and the effects of these agents on the induced frequency of gene mutations and chromosome aberrations have been studied. In general, the supplemental treatments which did alter the x-ray-induced mutation or chromosome rearrangement rate were found to increase this rate. However, the recent work of Thoday and Read,¹ Hayden and Smith,² and Giles and Riley³ provide evidence that by lowering the oxygen tension during x-ray exposure of plant material, the induced frequency of

chromosome aberrations is greatly reduced. In view of the implications of this finding, it seemed advisable to determine if a lowered oxygen concentration reduces the radiation damage to the genes in animal material.

Experimental Methods.—The induction of sex-linked, recessive, lethal mutations was investigated in the Oregon-R strain of *Drosophila melanogaster*. Wild-type males of this strain (from one to three days in age) were treated with x-rays in the manner described below and then immediately mated in a culture bottle to virgin females of the Muller-5 stock ($sc^{s1}B$ In-S w^a sc^s)[4]. Seven to eight days after the time of treatment the parents were removed from the culture bottle. The F_1 females, all of which carry an irradiated X-chromosome, were aged with their brothers (Muller-5 males) for at least two days in a fresh culture bottle in order to increase the chance of insemination. Approximately the first 200 F_1 females which hatched from the parent culture were separated individually after aging into shell vials containing one or two Muller-5 males. A preliminary examination was made of the F_2 offspring for the presence of wild-type males by viewing the flies inside the culture vial with a binocular dissecting microscope. If one or more wild-type males were observed, the X-chromosome being tested was classified as a chromosome with no lethal mutations. On the other hand, if no wild-type males were observed in the preliminary examination of the F_2 flies, the culture was set aside until all the offspring had hatched and then the flies were anesthetized and classified. If this examination verified the absence of wild-type males, a further test of this lethal-bearing X-chromosome was made by mating in a shell vial three of the F_2 females which were heterozygous for the Muller-5 chromosome to five of their Muller-5 brothers. The F_3 offspring were counted to substantiate the presence of a lethal-bearing chromosome.

Because of the intergradation between mutations that have a full lethal effect and those which are semilethal, the classification of a mutation as a lethal mutation is arbitrary. An X-chromosome was classified as having one or more lethal mutations if the following criterion was valid: no wild-type males in the F_2 generation and 5% or less males of this type in the F_3. Less than 2% of the F_2 cultures with no wild-type males contained less than twelve Muller-5 males. These were classified as lethals only if 15 or more offspring were present; otherwise, they were classed as failures.

X-radiation was administered to the flies by means of a General Electric, Maximar Model, 250-kvp. unit which contained a self-rectifying Coolidge tube with a tungsten target. The inherent filtration was equivalent to 3 mm. of aluminum. In all the experiments 250-kvp. x-rays, produced by a 15-ma current, were used. This gave a dosage rate of about 125 r per minute at the target distance of 61.5 cm. The dosage rate was kept constant in all experiments.

During irradiation one group of flies was maintained in a continuous flow of oxygen gas and another group in a continuous flow of nitrogen gas; both groups being kept at a known constant temperature by means of the apparatus to be described. The gases, obtained from commercial cylinders, were passed through copper coils which were immersed in a water-bath containing either tap water or a mixture of tap water and ice. A beaker with two pairs of small side arms was also partially immersed in this water-bath. The gas, after passing through the copper coils, entered the beaker by means of one of the side arms. Between this side arm and the one on the opposite side of the beaker, was stretched a rubber balloon (open at both ends) which contained the flies enclosed in a gauze capsule. After the gas passed over the flies, it was conducted through copper tubing from the beaker to the outside of the water-bath container. Two sets of copper coils and four side arms on the beaker made possible the simultaneous exposure to x-rays of one group of flies in oxygen and another group in nitrogen, both groups being maintained at the same temperature. The top of the beaker, which extended just above the surface of the water-bath, was covered by a lucite lid and the radiation was administered to the flies through this window. In addition to the four small side arms previously mentioned, there was one large side arm on the beaker which extended from the beaker to the outside of the water-bath. This side arm allowed insertion of a thimble chamber into the beaker at the location where the flies received the radiation and also made possible the insertion of a thermometer into the beaker for temperature measurements.

The treatment procedure may be summarized as follows. The irradiation apparatus was placed underneath the target port and the dosage being delivered was determined by placing the thimble chamber into the beaker. The thimble chamber and the electrometer used had recently been standardized. The calculated dosage to be given was obtained by averaging the five dosimeter readings taken. About 150 Oregon-R males were placed in each of two gauze capsules and each capsule inserted into one of the balloons. The gases were then passed across the flies and ten minutes later the temperature inside the beaker was checked and the x-ray exposure begun. After the time necessary for the desired dosage had elapsed, the males were removed from the gas and mated immediately to Muller-5 females.

Experimental Results.—The data were gathered by means of 31 individual experiments of the type described above. Chi-square tests of homogeneity of the three or more experiments conducted with a given gas, temperature and dosage show no indication of heterogeneity among the individual experiments. Therefore, it is permissible to lump the results from the individual experiments in which the flies received the same treatment. The lumped data showing the effects of oxygen concentration, temperature

and dosage on the frequency of induced lethal mutations are compiled in table 1. The striking reduction in the number of lethal mutations induced when the flies are maintained in a nearly oxygen-free atmosphere is clearly evident from this table and the curves presented in figure 1. Chi-square values obtained by testing the independence of the gas used on the mutation frequency are tabulated in the seventh column of this table. The reduction in the mutation rate in the nitrogen series is highly significant ($P < 0.01$) except in the experiments in which the flies were given 1000 r

TABLE 1

EFFECT OF O_2 CONCENTRATION, TEMPERATURE AND DOSAGE ON LETHAL MUTATION RATE

DOSAGE	TEMPERATURE RANGE	GAS	NO. CHROMOSOMES TESTED	NUMBER LETHALS	PER CENT LETHALS	x^2 GAS	x^2 TEMP.
Control	0.5– 1.0	O_2	602	1	0.2		...
	0.5– 1.0	N_2	608	2	0.3	. .	
	26.0–28.5	O_2	607	1	0.2		...
	26.0–28.5	N_2	605	1	0 2	.	
1000 r	1.5– 3.0	O_2	600	33	5 5		O_2 1.6
	1.5– 3.0	N_2	582	14	2.4	7.4	
	22.0–28.0	O_2	605	24	4.0		N_2 0.0
	22.0–28.0	N_2	594	14	2.4	2 5	
3000 r	1.0– 3.0	O_2	485	88	18.1		O_2 7.8
	1.0– 3 0	N_2	767	54	7 0	36.4	
	26.0–28 2	O_2	603	73	12.1		N_2 0.1
	26 0–28 2	N_2	775	56	7.2	9.0	
5000 r	1.0– 3.0	O_2	105	24	22.9		O_2 1.2
	1.0– 3.0	N_2	1206	119	9.9	40 3	
	23.0–29.0	O_2	429	78	18 2		N_2 3.8
	23 0–29.0	N_2	1523	118	7.7	16 8	

units and kept at the warm temperature. There seems no reason to doubt that more extensive experiments conducted at this dosage and temperature would provide a statistically significant difference. The results assembled in table 1 also furnish direct evidence, which substantiates the claim of some previous investigators,[5] that more mutations are induced when the flies are kept at a near freezing temperature than are induced at room temperature. The chi-square values obtained from tests of independence of temperature on mutation rate are tabulated in the last column of table 1.

At all three dosage levels more mutations are produced at the lower temperature when the flies are irradiated in an oxygen atmosphere, but the difference is statistically highly significant only in the 3000 r series.   In the nitrogen-treated flies, on which more extensive data are available at the higher dosages, the temperature difference causes no apparent alteration in the mutation rate.   At 5000 r units the chi-square value is on the borderline of the conventional 5% level of significance.   These results seem to indicate that the "temperature effect" is actually an effect of altered oxygen tension in the sperm possibly caused by differences in oxygen solubility
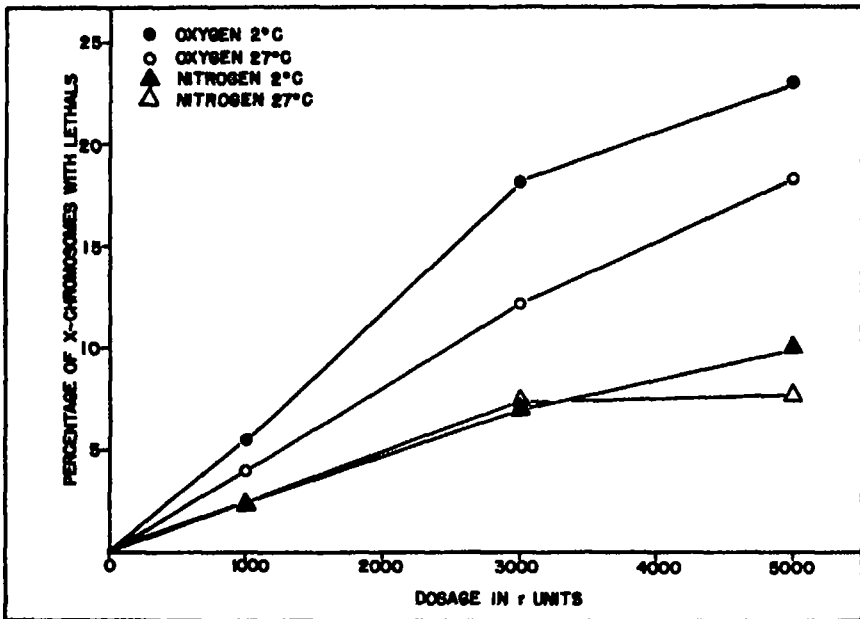
FIGURE 1

X-ray dosage curves for the lethal mutations induced in *Drosophila* irradiated in oxygen or nitrogen gas at 2° and 27° C.

and rate of respiration at the two temperatures.   It should be noted that the control experiments conducted without irradiation are not sufficiently large to give any reliable information concerning a possible relation between oxygen tension and spontaneous mutation rate.

It is evident from the figures presented in table 1 that at the higher dosages fewer chromosomes were tested in the oxygen-treated flies than in the nitrogen series.   Although a large number of experiments were conducted with the flies in oxygen, only a relatively small number of F_1 offspring were produced from the males which had been maintained in this

gas during irradiation.   The reduced number of offspring in the oxygen as
compared to the nitrogen series could possibly be due to any of four fac-
tors: (1) reluctance of the males to mate, (2) sperm immotility,[*] (3) lowered
viability of the treated males, or (4) increase in the frequency of dominant
mutations and chromosome aberrations which are lethal.   Experiments
were undertaken to determine which of these factors was effective.   After
irradiation of the Oregon-R males with 5000 r units at 27°C., sixty matings

DAYS AFTER TREATMENT
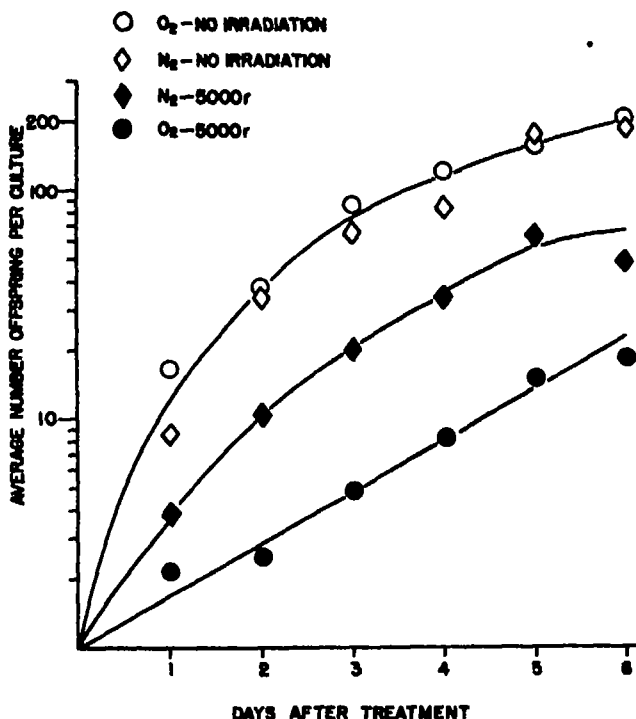
FIGURE 2

Relationship between the oxygen concentration at time of irra-
diation and the number of offspring produced.   On the abscissa is
plotted the number of days after treatment during which the
females were allowed to deposit eggs.

were made;   each mating consisting of three males treated in oxygen and
five Muller-5 females placed together in a shell vial.   Another 60 similar
matings were made using the males which had been exposed in an atmos-
phere of nitrogen.   Immediately after treatment the males in both series
were checked to see if any had been killed during irradiation.   Every 24
hours after exposure, the number of males living in each culture was ob-
served;   also, ten cultures were selected at random in each gas series and the
reproductive tracts of the five females in each culture were dissected out

and examined for the presence and motility of sperm. This procedure was repeated every 24 hours after treatment for six days. The number of offspring produced by each culture was counted. This gives an estimate of the number of offspring per culture produced from the eggs laid during the first 24, 48, 72, etc., hours after treatment.

The smaller number of offspring per culture produced by males which were exposed to 5000 r units of x-rays while in oxygen as compared with nitrogen can be seen in figure 2. Since the number of progeny in the two gas series is the same when the parent males are exposed to the gases without radiation, it is obvious that this effect must be caused by a difference in the biological action of the radiation on the flies in the two gases.

Only one case was observed of immotile sperm in the reproductive tract of the female and no evidence was obtained of any difference in the number
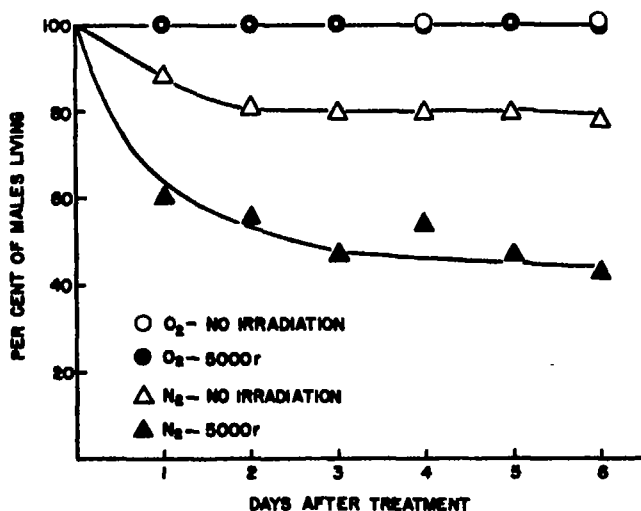
FIGURE 3

Relationship between the oxygen concentration at time of irradiation and the viability of the treated males.

of females inseminated; between 90 and 100% of the females were inseminated in both series from the first day on. These observations eliminate the possibility that the first two factors listed above are effective. The data presented in figure 3 indicate that male viability is not an important factor since it acts in the opposite direction. About one-half of the males exposed in nitrogen die during the first few days, while the viability of the flies in oxygen is not affected over the course of the experiment. Incidentally, it appears from a comparison of the two nitrogen curves in figure 3 that the differential killing is not only caused by the gas alone but is enhanced by the radiation. These findings then provide evi-

dence that the difference in the number of offspring produced from males treated in the two gases must be attributed to a reduction in the frequency of induced dominant lethal mutations when the flies are irradiated in nitrogen. Further evidence, based on the smaller number of failures of the F_1 matings in the nitrogen series, also indicates a lowering of the frequency of dominant sterility mutations in the absence of oxygen.

Discussion.—It appears reasonable to attribute the reduction in the frequency of mutations induced in the male flies irradiated in a nitrogen atmosphere to a lowered oxygen tension in their sperm. It must be assumed then that the nitrogen acts as an inert gas in so far as this effect is concerned and, also, the assumption must be made that, by greatly reducing the oxygen concentration surrounding the flies, the oxygen tension in the sperm is lowered. Since Giles and Riley[1] found that the reduction, as compared to air or oxygen, in the number of induced chromosome aberrations was evident when the *Tradescantia* influorescences were irradiated in helium and argon as well as nitrogen, there is no reason to doubt the first assumption. The second assumption seems most likely since the gas transport to the body tissues in small insects with a tracheal respiratory system takes place almost entirely by diffusion.[7]

The higher frequency of mutations induced in the flies maintained in oxygen at the low temperature is in agreement with the results expected if oxygen is an agent influencing the radiosensitivity of genes. A lowering of the temperature would affect at least two processes in such a way that the oxygen tension in the cell would be increased: (1) the solubility of oxygen in water is higher at colder temperatures, (2) with the low rate of metabolism associated with the cold temperature, the oxygen gradient across the cell membrane would be less than at the warm temperature. This would, in turn, raise the oxygen concentration within the cell. It should be noted, however, that the rate of diffusion of the oxygen would be lower at the colder temperature.

If the oxygen tension within the cell is a factor in determining the sensitivity of genes to x-rays, the question arises as to its mode of action. At the present time any answer to this question is purely speculative. Increased amounts of dissolved oxygen in the cellular fluids being irradiated would form more of the free radicals which act as strong oxidizing agents and which are known to be produced in water by x-radiation. Barron, *et al.*,[8] have published evidence which indicates that it is the oxidizing properties of these radicals which cause the x-ray inhibition of the sulfhydryl enzymes. If such enzymes are necessary in the process of reduplication of particular genes, their inhibition could produce a lethal mutation. On the other hand, recent experiments[9] indicate that organic peroxides, which have rather poor oxidizing properties, are effective mutagenic agents. Thus upon irradiation of cells with high oxygen tensions, the increased amounts

of organic peroxides formed would serve to increase the mutation rate. To date, there is no evidence on which the validity of either mode of action can be established.

Summary.—There is a striking reduction in the number of recessive sex-linked lethal mutations induced in *D. melanogaster* males when they are exposed to x-rays while in an atmosphere of low oxygen concentration. Although an increased number of mutations were induced in flies irradiated in oxygen at 2°C. over those treated in oxygen at 27°C., this increase is not due to the temperature *per se* but rather it is apparently caused by a higher oxygen tension within the irradiated sperm at the lower temperature. Additional evidence also indicates that fewer dominant lethal mutations and chromosome aberrations are induced in flies maintained in a near oxygen-free atmosphere during irradiation.

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† Contribution No. 32 from the Department of Zoology and Entomology.

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[4] For a complete description of the Muller-5 method see Spencer, W. P., and Stern, C., *Genetics*, 33, 43 (1948).

[5] For a review of this subject see Baker, W. K., *Ibid.*, 34, 167 (1949).

[6] There is a remote possibility that the oxygen gas might affect the ability of the sperm to fertilize the eggs without disturbing their motility.

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SOME REMARKS ON THE INFINITE DE SITTER SPACE

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One can define de Sitter space as a four-dimensional space with the following two properties. First, it is invariant under the operations of a transitive ten-parametric group. Four of the infinitesimal operators of this group are usually made to correspond to the components of the energy-momentum vector, the other six to the angular momentum tensor. Second, the subgroup of this ten-parametric group which leaves a given point of the space invariant must be isomorphic to the ordinary homogeneous Lorentz

group. This last point ensures that the neighborhood of any point of these de Sitter spaces behaves like the flat space of special relativity (Minkowski space).

The best known examples of de Sitter space in the above sense are, first, the flat space of special relativity, and, second, the ordinary de Sitter spaces. In the former case the ten-parametric group is the inhomogeneous Lorentz group, i.e., the union of Lorentz transformations with the displacements in the four directions of space and time. This space can be regarded as a special case of the ordinary de Sitter spaces. The latter can be represented as the four-dimensional surface

$$x_1{}^2 + x_2{}^2 + x_3{}^2 + x_4{}^2 - x_5{}^2 = a^2 \tag{1}$$

in five-dimensional space. The ten-parametric group is, in this case, the $4 + 1$ dimensional homogeneous Lorentz group, i.e., the group of linear homogeneous transformations which leave the form (1) invariant. Given an arbitrary point of this space, e.g., $x_4 = a$, $x_1 = x_2 = x_3 = x_5 = 0$, the subgroup which leaves it invariant is indeed the ordinary homogeneous $(3 + 1$ dimensional) Lorentz group: it is the group which leaves $x_1{}^2 + x_2{}^2 + x_3{}^2 - x_5{}^2$ invariant. One sees that the ordinary de Sitter space is in a sense more symmetric than the Minkowski space because all the infinitesimal elements of its group are on the same footing. Four of the infinitesimal elements of Minkowski space, i.e., the translations, are distinguished inasmuch as they form a four-parametric commutative subgroup. The Minkowski space can be obtained from the ordinary de Sitter space by setting $a = \infty$ in (1) and restricting the space with the conditions x_1, x_2, x_3, $x_5 \ll a$, $x_4 \approx a$, i.e., to the neighborhood of a point.

The de Sitter space (1) has a finite extension in the sense that, given any point P (e.g., the point $x_4 = a$; $x_1 = x_2 = x_3 = x_5 = 0$) and any time-like direction in that point (e.g., the intersection of (1) with the x_4x_5 plane), the geodesics through P, which are perpendicular to the chosen time-like direction, are finite. Three such geodesics in the above case are the intersections of (1) with the x_1x_4, x_2x_4, x_3x_4 planes. These geodesics form circles in the underlying five-dimensional space and return to P after a distance $2\pi a$. Neither is it possible to make this space infinite by considering the point at the distance $2\pi a$ on the geodesic which is, for example, in the x_1x_4 plane, to be different from the original point P. The reason is that one can deform the geodesic in question continuously into a curve of zero length by tilting the plane x_1x_4 about the $x_4 = a$, $x_2 = x_3 = x_5 = 0$ axis in the x_2 direction, i.e., by considering the intersection of (1) with the planes

$$x_2 = x_5 = 0, \qquad x_3 = (x_4 - a)\, tg\vartheta \tag{2}$$

and increasing ϑ from 0 to $\pi/2$. Hence the ordinary de Sitter space (1) is essentially finite in extension.

The question arises, therefore, whether or not there is a really infinite de Sitter space. The purpose of the present note is to point out that there is. It is formed by the four-dimensional surface

$$x_1{}^2 + x_2{}^2 + x_3{}^2 - x_4{}^2 - x_5{}^2 = - a^2 \tag{3}$$

in five-dimensional space. Its group is the $3 + 2$ dimensional Lorentz group, i.e., the group of linear homogeneous transformations which leave (3) invariant. Choosing an arbitrary point P on (3), e.g., the point $x_4 = a, x_1 = x_2 = x_3 = x_5 = 0$, the subgroup which leaves this point invariant is again the ordinary homogeneous Lorentz group.

The space (3) is contained in the enumerations of both Friedmann[1] and Robertson.[2] It is clearly infinite. If one considers the point P as above, and as time-like direction the intersection of (3) with the $x_4 x_5$ plane, geodesics through P which are perpendicular to the chosen time-like direction are

$$x_4 = a \operatorname{Ch}\varphi; \quad x_\alpha = a \operatorname{Sh}\varphi; \quad \text{all other } x = 0 \tag{4}$$

with $\alpha = 1, 2, 3$. These have infinite lengths and are completely space-like (i.e., there is no time-like line through any two of their points).

By suppressing the coordinates x_2 and x_3, one can make a diagram of the surface (3) which looks exactly like the corresponding diagram obtained by setting $x_2 = x_3 = 0$ in (1). However, the geodesics which are space-like if the diagram is considered to represent the finite de Sitter space, are time-like if it is considered to be a representation of the infinite de Sitter space (3). The diagram, figure 1, shows indeed that the space of (3) is infinite but also shows that its time is finite or, rather, periodic.

Geometrically, this is not necessarily true because one can replace the space (5) with its covering space. This can be done, for instance, by introducing new variables

$$x_4 = \rho \cos \vartheta; \quad x_5 = \rho \sin \vartheta \tag{5}$$

and not identifying the points the ϑ of which differs by 2π. In contrast to the de Sitter space (1), this is a possible procedure because the different signature of the suppressed coordinates x_2 and x_3 in (3) prevents the line (5) in (3) to be contracted. However, it is doubtful whether in *non quantum* theory the covering surface differs essentially from the original surface because all geodesics (which are the intersections of (3) with planes through the origin $x_1 = x_2 = x_3 = x_4 = x_5 = 0$) which start from a point P will return to that point. As a result, at least in the approximation in which the world lines are geodesics, the world is periodic with the period $2\pi a$ (actually with the period πa) no mater what the initial distribution of the masses is.

The purpose of the present note is to point out that there is an essential difference between the properties of the infinite de Sitter space as viewed from the *quantum* rather than the *non quantum* theoretic point of view. The reason is that not all wave functions have to be periodic in the ϑ of (5) with a period 2π, in fact they can differ by any factor of modulus 1 between two points of the covering space which correspond to the same point P of the space (3). This is equivalent with the statement that the wave functions, if regarded as functions of, say, x_1, x_2, x_3, ρ and ϑ, need not be periodic in ϑ with a 2π period.

There is one further remarkable fact about wave functions in either the space of (3) or in its covering space. The wave functions of "elementary systems" in (3) are given by the irreducible representations of the group of (3), that is the $3 + 2$ dimensional Lorentz group. Bargmann and G. W. Whitehead[3] have determined the topology of this group: It consists of the direct product of a three-dimensional rotation group (2 parameters), of a six-dimensional euclidean space (6 parameters) and of a two-dimensional rotation group (1 parameter). The representations up to a factor of this
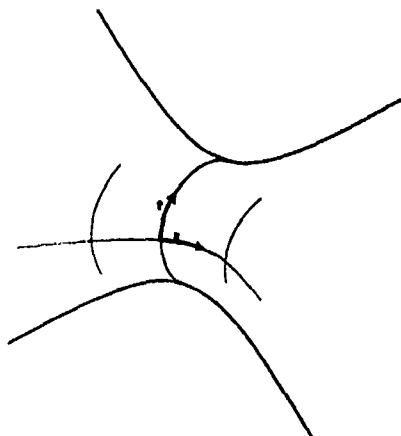
FIGURE 1

group have, therefore, one- or two-valued character, corresponding to the presence of the three-dimensional rotation group in the topology of the group of (3). They have, furthermore, one of an infinite number of characters corresponding to the infinitely many-valued representations of the two-dimensional rotation group. These correspond to the possibilities of representing the subgroup of rotations in the $x_4 x_5$ plane by any of the matrices $e^{i\kappa\varphi}$, where κ is not necessarily an integer but can be, in fact, entirely arbitrary. In every irreducible representation of the whole group only those representations of the subgroup will occur in which the fractional part of κ is the same, i.e., which differ by an integer. As a consequence, the fractional part of κ is, in the space (3), an integral of motion, just as the integer or half integer character of the spin is an integral of motion in ordinary quantum theory.

If we consider, on the other hand, the physical space as the covering space of (3), the existence of the different types of representations will remove the periodic character of the world. The extra integrals of motion will remain, however, because those transformations of the covering space,

which leave the subspace (3) invariant, commute with all symmetry operations of the group of the covering space.

There is no obvious physical interpretation which one could give these integrals of motion. The fact that the topological structure of the world influences in quantum theory the integrals of motion remains remarkable enough to be recorded.

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THE EFFECT OF SEMINAL PLASMA ON FERTILIZED RABBIT OVA*

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Seminal plasma, the secretion of the male accessory glands, is the natural fluid for the transportation and survival of spermatozoa. This fluid, however, has a harmful effect on fertilized ova as demonstrated in the following experiments.

Methods.—Fertilized rabbit ova (in 2 cells) were flushed out with rabbit serum from the fallopian tubes of superovulated does about 15 hours after ovulation. The seminal plasma was obtained by centrifugation of fresh human, bull or rabbit whole semen. The seminal plasma of vasectomized rabbits without centrifugation was also used. The seminal plasma in undiluted form or after heat treatment, or diluted with fresh rabbit serum, was used to culture freshly recovered ova in a Carrel flask for 1 day at 38°C. To prevent infection, penicillin was added. Ordinarily, 2-celled ova, cultured in rabbit serum for 1 day, cleave into 12 to 16 cells or occasionally into 32 cells. In the present study, all ova which had divided into 7 cells or more were arbitrarily classified as normal, while those which showed cleavage of less than 7 were considered abnormal.

Results: Ovum Culture in Seminal Plasma.—Table 1 shows the results obtained when fertilized rabbit ova were cultured in seminal plasma of man, rabbit and bull, diluted with rabbit serum. The harmful effect of heterologous as well as homologous seminal plasma on rabbit ova is clearly shown. All of the ova disintegrated in undiluted rabbit or bull seminal plasma, and only 1 out of 16 cleaved normally in human seminal plasma. When bull or rabbit seminal plasma was diluted with 75% rabbit serum,

none of the ova divided normally in bull plasma, whereas half underwent cleavage in rabbit plasma. Thus, the strength or concentration of the harmful substance varies in different species in ascending order as follows: human, rabbit and bull.

The inhibition of cleavage and disintegration of ova in seminal plasma are not due to lack of nutrients, but to the presence of a harmful substance, because ova divided normally in 0.9% NaCl containing 25% rabbit serum. Since seminal plasma of both vasectomized and normal rabbits showed similar harmful effects (table 1), the inhibitory substance must be a component of the secretion of the accessory glands instead of a product of the spermatozoa.

TABLE 1

CLEAVAGE OF FERTILIZED RABBIT OVA CULTURED FOR 1 DAY AT 38°C. IN SEMINAL PLASMA VARIOUSLY DILUTED WITH RABBIT SERUM

SEMINAL PLASMA OF	DILUTED WITH RABBIT SERUM %	TOTAL NO. OF OVA	NO. SHOWING NORMAL CLEAVAGE
Man	0	16	1
	50	17	8
	75	14	9
	87.5	9	7
Rabbit	0	18	0
	50	22	0
	75	19	9
	87.5	10	6
Vasectomized rabbit	0	10	0
	50	9	0
	75	17	11
	87.5	16	12
Bull	0	17	0
	50	22	0
	75	21	0
	87.5	19	6

When rabbit ova were treated with undiluted rabbit seminal plasma for 10, 20, 40 or 80 minutes at 30°C. and then cultured in serum for 1 day, 40 out of 54 (74%) cleaved. It seems that, unlike the ovocidal factor present in the heterologous serum,[1] the harmful substance in the seminal plasma is not immediately lethal, but exerts its harmful effect after a longer period of time.

Characteristics of Ovocidal Seminal Plasma Factor.—Table 2 presents data obtained when seminal plasma was heated at 55°C. for various lengths of time and then used for ovum culture. The inhibitory effect of undiluted seminal plasma was not abolished by the heat treatment, but the cleavage percentages in the serum-heated plasma cultures suggest

some destruction by the heating of the inhibitory factor. This is emphasized by the complete failure of the ova to undergo cleavage in 50% serum-unheated plasma (data of table 1). In a subsidiary experiment, bull and rabbit seminal plasma were stored at 5°C. for 2 weeks and each was then mixed with equal volumes of blood serum. Cleavage of 75% of the ova cultured in this mixture suggests that the inhibitory factor is lost with time.

Rabbit ova were cultured at 38°C. or stored at 10°C. in rabbit seminal plasma or serum-seminal plasma mixtures for 1 day. Normal cleavage was not observed. Then the ova were cultured in undiluted serum for 1 day at 38°C. Table 3 presents the results. It is clear that the seminal plasma effect is most pronounced at body temperature. Since ovum

TABLE 2

CLEAVAGE OF RABBIT OVA CULTURED IN RABBIT SEMINAL PLASMA HEATED FOR VARIOUS LENGTHS OF TIME

SEMINAL PLASMA HEATED AT 55°C., MINUTES	DILUTED WITH RABBIT SERUM %	TOTAL NO. OF OVA	NO. SHOWING CLEAVAGE	CLEAVAGE, %
30	0	18	0	0
	50	26	6	23
120	0	15	0	0
	50	40	20	50

TABLE 3

CLEAVAGE OF RABBIT OVA CULTURED IN SERUM FOLLOWING CULTURE IN SEMINAL PLASMA FOR 1 DAY

TREATMENT BEFORE CULTURE		CLEAVAGE IN UNDILUTED SERUM AT 38°		
TEMP., °C.	DILUTED WITH SERUM, %	TOTAL NO. OF OVA	NO. SHOWING CLEAVAGE	CLEAVAGE, %
38	0	12	0	0
	50	21	4	19
10	0	19	5	26
	50	21	13	62

disintegration occurred only after the elapse of 80 minutes in undiluted seminal plasma at 30–38°C., the harmful effect involves either a slow process or the accumulation of metabolic products of the seminal plasma.[5] The ovocidal substance in diluted form may have inhibitory action because no ova cleaved in seminal plasma containing 50% serum at 38°C., but there was 19% cleavage when the ova were subsequently cultured in undiluted serum (table 3).

The ultrafiltrate of bull or rabbit seminal plasma diluted with 50% of serum does no obvious harm to the ova, suggesting that the harmful substance is of large molecular size. Seminal plasma contains about 10 times the amount of phosphorus contained in serum.[4] The high concentration of phosphorus compounds derived from the seminal vesicles[3] and the large

amount of phosphatase originating in the prostate glands[4] lead one to suspect that the harmful substance may be a heat labile, organic phospho-compound, emanating from these glands or derived from the metabolism of seminal plasma.

Rabbit blastocysts, recovered from the uterus at either 4 or 6 days after mating, were cultured in rabbit seminal plasma containing 50% of serum at 38°C. for 1 day, but no obvious evidence of damage was observed and ovum growth approximated that observed in control cultures. In contrast, it is interesting to note that 4- to 6-day rabbit blastocysts in the normal uterine environment are extraordinarily dependent on a hormone-labile nutrition, since ovariectomy[2] or estrogen administration[6] prevents their growth and development *in vivo*. Early rabbit ova, on the other hand, exhibit normal cleavage under these conditions which affect the blastocysts so adversely.[7] It is evident therefore that at their various developmental stages rabbit ova exhibit markedly different susceptibilities to factors affecting growth and development.

Summary.–Human, rabbit and bull seminal plasma causes the disintegration of fertilized rabbit ova and inhibits cleavage even when diluted with rabbit serum. The toxic factor appears to be most concentrated in bull seminal plasma and least so in human seminal plasma. The toxic factor is thermolabile, and disappears during prolonged storage at 5°C. Its typical action occurs after a measurable latent period which increases with increasing temperature.

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ABSOLUTE AND UNCONDITIONAL CONVERGENCE IN NORMED LINEAR SPACES

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1. Let B be a real Banach space and denote by $\|x\|$ the norm of an element x of B. The series

$$\sum_{\nu=1}^{\infty} x_\nu \quad (x_\nu \epsilon B, \, \nu = 1, 2, \ldots) \tag{1}$$

is called absolutely convergent if $\sum \|x_\nu\| < \infty$; it is called unconditionally convergent if the series $\sum y_\nu$ converges whenever the sequence $(y_\nu)_1^\infty$ is a rearrangement of the sequence $(x_\nu)_1^\infty$. An equivalent definition of unconditional convergence of (1) is obtained by requiring $\sum \pm x_\nu$ to be convergent for every choice of the signs. There are several other equivalent definitions; most of these have been discussed by T. H. Hildebrandt.[1]

It is clear that if B is of finite (linear) dimension then (1) is unconditionally convergent if and only if it is absolutely convergent. The problem of finding the spaces for which these two types of convergence are equivalent is mentioned by S. Banach.[2] The primary aim of this note is to settle this problem by proving the following result.

THEOREM 1. *The unconditionally convergent series coincide with the absolutely convergent series if and only if the space B is of finite dimension.*

Here the only non-trivial assertion is that, if B is of infinite dimension, there is a series (1), which is unconditionally but not absolutely convergent. It is easy to give examples of such series in Hilbert space and similar examples have been given[3] for all the usually encountered infinitely dimensional Banach spaces. Interesting partial results on the problem solved by Theorem 1 have been established by M. E. Munroe[4] and S. Karlin.[5] The two last mentioned papers treat also some related problems and give various consequences of Theorem 1.

Our method of proof yields not only Theorem 1 but also the following result.

THEOREM 2. *If B is of infinite dimension and $\sum c_\nu$ is any convergent series of positive terms, then there exists an unconditionally convergent series (1) satisfying $\|x_\nu\|^2 = c_\nu$ for $\nu = 1, 2, \ldots$.*

Applying this result with $c_\nu = \nu^{-1}[\log(1+\nu)]^{-2}$ we obtain:

COROLLARY: *If B is of infinite dimension then there exists an unconditionally convergent series (1) having the property that $\sum \|x_\nu\|^{2-\epsilon} = \infty$ for every $\epsilon > 0$.*

Theorem 1 is obviously an immediate consequence of this Corollary.

If B is a Hilbert space then $\sum \|x_r\|^2 < \infty$ for every unconditionally convergent series (1). Thus Theorem 2 and its Corollary are in a certain sense best possible results.

A result (Lemma 1) concerning convex bodies in Euclidean space is proved in section 2. In section 3 this lemma is used to prove Theorem 2, and remarks are made concerning its extension. In section 4 some geometrical properties of convex bodies are obtained from Lemma 1 and from the construction used to prove this lemma.

2. We consider the n-dimensional Euclidean space of points $U = (u_1, \ldots, u_n)$ and use the usual vector notation. We first prove our main lemma.

LEMMA 1. *Let C be a body[6] which is convex and has the origin O as center, and let r be an integer with $1 \leq r \leq n$. Then there are n points $A_1, \ldots, A_n$ on the boundary of C such that, if $\lambda_1, \ldots, \lambda_r$ are any r real numbers with $1 \leq r \leq n$, then the point $\lambda_1 A_1 + \lambda_2 A_2 + \ldots + \lambda_r A_r$ is in the body λC where*

$$\lambda^2 = \left[2 + \frac{r(r-1)}{n} \right] (\lambda_1^2 + \lambda_2^2 + \ldots + \lambda_r^2). \qquad (2)$$

Proof: We inscribe in C an ellipsoid with O as center having the largest possible n-dimensional volume. Since it is enough to establish the lemma for any affine transform of C, we may assume that this ellipsoid is the sphere S of unit radius.

We first show that after a suitable orthogonal transformation has been applied there will be r points $A_1, \ldots, A_r$ of contact of C with S, satisfying for $\rho = 1, 2, \ldots, r$

$$\left. \begin{aligned} A_\rho &= (a_{\rho 1}, a_{\rho 2}, \ldots, a_{\rho \rho}, 0, \ldots, 0), \\ a_{\rho 1}^2 &+ \ldots + a_{\rho(\rho-1)}^2 = 1 - a_{\rho\rho}^2 \leq \frac{\rho-1}{n}. \end{aligned} \right\} \qquad (3)$$

For $r = 1$ this is clear; assuming it for $r = m - 1 < n$ we prove it for $r = m$. The ellipsoid

$$(1 + \epsilon)^{n-m+1}(u_1^2 + \ldots + u_{m-1}^2) +$$
$$(1 + \epsilon + \epsilon^2)^{-m+1}(u_m^2 + \ldots + u_n^2) \leq 1, \qquad (\epsilon > 0) \quad (4)$$

has a volume larger than that of S. Hence there is a point $A = A(\epsilon) = (a_1, \ldots, a_n)$ on the boundary of C in the ellipsoid (4). But, since A being on the boundary of C is not inside the unit sphere, we have $a_1^2 + \ldots + a_n^2 \geq 1$. It follows that A satisfies

$$[(1 + \epsilon)^{n-m+1} - 1](a_1^2 + \ldots + a_{m-1}^2) +$$
$$[(1 + \epsilon + \epsilon^2)^{-m+1} - 1] (a_m^2 + \ldots + a_n^2) \leq 0. \quad (5)$$

If $\epsilon \to 0$ through a suitable sequence of positive numbers the corresponding sequence $A(\epsilon)$ will converge to a point A_m. It is clear from (4) that A_m is

a point of contact of S and the boundary of C, while from (5) we have in the limit

$$(n - m + 1) (a_{m1}{}^2 + \ldots + a_{m(m-1)}{}^2) +$$
$$(-m + 1) (a_{mm}{}^2 + \ldots + a_{mn}{}^2) \leq 0. \quad (6)$$

By a suitable orthogonal transformation of the variables $u_m, \ldots, u_n$, leaving the points $A_1, \ldots, A_{m-1}$ invariant we may make the last $n - m$ coordinates of A_m vanish. Then, using (6) and the equation $a_{m1}{}^2 + \ldots + a_{mn}{}^2 = 1$ we obtain (3) with $\rho = m$. Thus (3) is proved for $\rho = 1, 2, \ldots, n$

Let $\lambda_1, \ldots, \lambda_r$ be any real numbers. By (3) the square of the distance from O to the point $\lambda_1 A_1 + \ldots + \lambda_r A_r$ is

$$\sum_{\sigma=1}^{r} \left(\sum_{\rho=\sigma}^{r} \lambda_\rho a_{\rho\sigma} \right)^2 \leq \sum_{\sigma=1}^{r} \left[2\lambda_\sigma{}^2 a_{\sigma\sigma}{}^2 + 2 \left(\sum_{\rho=\sigma+1}^{r} \lambda_\rho a_{\rho\sigma} \right)^2 \right]$$
$$\leq \sum_{\sigma=1}^{r} 2 \left[\lambda_\sigma{}^2 a_{\sigma\sigma}{}^2 + \left(\sum_{\rho=\sigma+1}^{r} \lambda_\rho{}^2 \right) \left(\sum_{\tau=\sigma+1}^{r} a_{\tau\sigma}{}^2 \right) \right]$$
$$= 2 \sum_{\rho=1}^{r} \left[a_{\rho\rho}{}^2 + \sum_{\tau=1}^{r} \sum_{\sigma=1}^{\min.\,(\rho-1,\,\tau-1)} a_{\tau\sigma}{}^2 \right] \lambda_\rho{}^2.$$

But by (3), the last expression is less than or equal to

$$2 \sum_{\rho=1}^{r} \left(1 + \sum_{\tau=1}^{r} \frac{\tau - 1}{n} \right) \lambda_\rho{}^2 = \left[2 + \frac{r(r - 1)}{n} \right] \sum_{\rho=1}^{r} \lambda_\rho{}^2 = \lambda^2.$$

Thus the point $\lambda_1 A_1 + \ldots + \lambda_r A_r$ is contained in the sphere λS and so is contained in the body λC. This proves the lemma.

3. Before we prove Theorem 2 it is convenient to obtain the following consequence of Lemma 1.

LEMMA 2. *Let B be a Banach space of infinite dimension and let $c_1, \ldots, c_r$ be any given positive numbers. Then there exist points $x_1, \ldots, x_r$ in B with $\|x_\rho\|^2 = c_\rho$ for $\rho = 1, \ldots, r$ and such that, if $\sum'$ denotes the sum over any subset of the numbers $1, \ldots, r$, then*

$$\|\textstyle\sum' x_\rho\|^2 \leq 3 \sum' c_\rho. \quad (7)$$

Proof: Write $n = r(r - 1)$. As B is of infinite dimension we can choose n linearly independent elements $z_1, \ldots, z_n$. Then the points $U = (u_1, \ldots, u_n)$ with $\|u_1 z_1 + \ldots + u_n z_n\| \leq 1$ form a convex body C with the origin as center in n-dimensional Euclidean space. Let $A_1, \ldots, A_r$ be the points given by Lemma 1. Writing $A_\rho = (a_{\rho 1}, \ldots, a_{\rho n})$, we put

$$x_\rho = c_\rho{}^{1/2}(a_{\rho 1} z_1 + \ldots + a_{\rho n} z_n), \qquad \rho = 1, \ldots, r.$$

Then, as $A_1, \ldots, A_r$ are on the boundary of C, we have $\|x_\rho\|^2 = c_\rho$, for $\rho = 1, \ldots, r$. Further, as the point $\sum' c_\rho{}^{1/2} A_\rho$ is in λC where $\lambda^2 = 3 \sum' c_\rho$, it follows that (7) is satisfied. This proves the lemma.

Proof of Theorem 2:   Choose a strictly increasing sequence $n_1 = 0, n_2, n_3,$ ... of integers such that the series

$$\sum_{r=1}^{\infty} \left(\sum_{\nu=n_r+1}^{n_{r+1}} c_\nu \right)^{1/2}$$

is convergent.   By Lemma 2 we can choose x_ν for $n_r < \nu \leq n_{r+1}$ so that $\|x_\nu\|^2 = c_\nu$ and $\|\sum^{(r)} x_\nu\|^2 \leq 3 \sum^{(r)} c_\nu$, the sum $\sum^{(r)}$ being taken over any subset of the integers ν with $n_r < \nu \leq n_{r+1}$.   Let $\sum y_\nu$ be any rearrangement of the series $\sum x_\nu$.   Let $\epsilon > 0$ be given.   Choose r so large that

$$\sum_{\rho=r}^{\infty} \left(\sum_{\nu=n_\rho+1}^{n_{\rho+1}} c_\nu \right)^{1/2} < \frac{\epsilon}{2}.$$

Choose p so large that the sum $\sum_{\nu < p} y_\nu$ includes all the terms x_ν with $\nu \leq n_r$. Then for any $q > p$ we have

$$\left\| \sum_{\nu=p}^{q} y_\nu \right\| \leq \sum_{\rho=r}^{\infty} \left\| \sum^{(\rho)} x_\nu \right\| \leq \sum_{\rho=r}^{\infty} \left(3 \sum_{\nu=n_{\rho+1}}^{n_{\rho+1}} c_\nu \right)^{1/2} < \epsilon.$$

Since B is complete it follows that $\sum y_\nu$ is convergent.   As this is true for every rearrangement of $\sum x_\nu$, the series $\sum x_\nu$ is unconditionally convergent and Theorem 2 is proved.

We note that the completeness of B was used only to deduce the convergence of $\sum y_\nu$ from its Cauchy convergence.   Hence we have (with obvious meaning of unconditional Cauchy convergence)

THEOREM 3.   *Let N be an infinitely dimensional normed linear space over the reals and $\sum c_\nu$ be any convergent series of positive numbers.   Then there exists an unconditionally Cauchy convergent series $\sum x_\nu$ of elements of N satisfying $\|x_\nu\|^2 = c_\nu (\nu = 1, 2, \ldots)$.   In particular there exist such series with $\sum \|x_\nu\| = \infty$.*

Since a complex Banach space contains a real one, it is clear that Theorems 1 and 2 hold for complex Banach spaces.   A similar remark applies to Theorem 3.

4.   In this section we prove some geometrical results.   The first result shows that Lemma 1 can be considerably improved in the special case where $r = n$ and $\lambda_1 = \pm 1, \ldots, \lambda_n = \pm 1$.

THEOREM 4.   *Let C be a convex body with the origin O as center.   Then there are points $P_1, \ldots, P_n$ on the boundary of C such that all the 2^n points $\pm P_1 \pm \ldots \pm P_n$ are in the body $2n^{1/2}C$.*

Proof: For $n > 1$ let q, r, s be the non-negative integers defined by

$$r(r-1) \leq 2n < r(r+1), \qquad n = qr + s, \qquad s < r. \quad (8)$$

Let $A_1, \ldots, A_r$ be the points thus denoted in Lemma 1 and for $t = 1, 2, \ldots, n$ put $P_t = A_{\nu(t)}$, where $\nu(t) \equiv t \pmod{r}$ and $1 \leq \nu(t) \leq r$.

Then all 2^n points $\doteq P_1 \doteq \ldots \doteq P_n$ are of the form $\lambda_1 A_1 + \ldots + \lambda_r A_r$, where the integers λ_r satisfy the inequalities

$$|\lambda_r| \leq q + 1 \text{ for } 1 \leq \nu \leq s, \qquad |\lambda_r| \leq q \text{ for } s < \nu \leq r.$$

Hence, by Lemma 1 all the 2^n points considered are in the body μC where

$$\mu^2 = \left[2 + \frac{r(r-1)}{n} \right] \sum_{r=1}^{r} \lambda_r^2 \leq 4 \left[s(q+1)^2 + (r-s)q^2 \right].$$

Taking account of (8) it is easily checked that $s(q+1)^2 + (r-s)q^2 < n^{1/2}$ for $n > 1$. The theorem being obvious for $n = 1$, is thus completely proved.

Remark: It is of some interest to find the exact dependence of μ on n. Our method, though capable of improving the constant 2 in this theorem, cannot improve the power in the estimate $\mu < 2n^{1/4}$. When C is a sphere then an enlargement by the factor $n^{1/2}$ is sufficient. Perhaps this is generally true, but we cannot prove it for $n \geq 3$.

We give a proof for $n = 2$ in the hope that it may be generalized to other values of n. Let B be the two-dimensional Banach space whose unit sphere is C. Given any point P_1 in this space with $\|P_1\| = 1$ there exists, by continuity, a point P_2 satisfying $\|P_2\| = 1$ and $\|P_1 + P_2\| = \|P_1 - P_2\|$. Let α denote this common norm, then also $\|\pm P_1 \doteq P_2\| = \alpha$. Now put $Q_1 = (P_1 + P_2)/\alpha$, $Q_2 = (P_1 - P_2)/\alpha$, then $\|Q_1\| = \|Q_2\| = 1$ and $\|\doteq Q_1 \doteq Q_2\| = 2/\alpha$. Since min.$(\alpha, 2/\alpha) \leq 2^{1/2}$ the proof is completed.

The following results are simple consequences of the construction used in proving Lemma 1. We include them since they seem to be of some geometrical interest.

THEOREM 5A. *Let C be a convex body with the origin as center. Then there is an ellipsoid $\mathcal{E}$ contained in C and a parallelopiped $\mathcal{P}$ containing C with volumes $V(\mathcal{E})$ and $V(\mathcal{P})$ satisfying*

$$\frac{V(\mathcal{P})}{V(\mathcal{E})} \leq \frac{2^n}{J_n} \left(\frac{n^n}{n!} \right)^{1/2}, \tag{9}$$

where J_n is the volume of the unit n-dimensional sphere.

Proof: Take $\mathcal{E}$ to be an ellipsoid with O as center having the largest possible volume. As in the proof of Lemma 1, we may suppose without loss of generality that $\mathcal{E}$ is the unit sphere S and denote by $A_1, \ldots, A_n$ points of contact of C and S satisfying (3). As C contains S the only tac-plane to C at A_r is the plane $a_{r1}u_1 + \ldots + a_{rr}u_r = 1$. Thus C is contained in the parallelopiped $\mathcal{P}$ defined by $|a_{r1}u_1 + \ldots + a_{rr}u_r| \leq 1, r = 1, 2, \ldots, n$. By (3) the volume of $\mathcal{P}$ satisfies

$$V(\mathcal{P}) = 2^n |a_{11}a_{22} \ldots a_{nn}|^{-1} \leq 2^n \left(\frac{n^n}{n!} \right)^{1/2} = 2^n \left(\frac{n^n}{n!} \right)^{1/2} \frac{V(\mathcal{E})}{J_n}.$$

THEOREM 5B.   *Under the conditions of Theorem 5A there is an ellipsoid $\mathcal{E}$ containing C and an "octahedron" Θ contained in C, with*

$$\frac{V(\mathcal{E})}{V(\Theta)} \leq (1/2)^n J_n (n!n^n)^{1/n}. \tag{10}$$

Proof: The result follows immediately by application of Lemma 3 to the body K which is the polar reciprocal of C.

THEOREM 6.   *Let C and K be convex bodies with the origin as center, which are polar reciprocal. Then their volumes satisfy*

$$\frac{2^n J_n}{(n!n^n)^{1/n}} \leq V(C) \cdot V(K) \leq 2^n J_n \left(\frac{n^n}{n!}\right)^{1/n}. \tag{11}$$

Proof: By Lemma 3 we may suppose without loss of generality that C contains the unit sphere S and is contained in a parallelopiped $\mathcal{P}$ with volume $V(\mathcal{P})$ satisfying

$$V(\mathcal{P}) \leq 2^n \left(\frac{n_n}{n!}\right)^{1/n}. \tag{12}$$

Then K is contained in S and contains an "octahedron" Θ with

$$V(\Theta) \geq \frac{2}{(n!n^n)^{1/n}} \tag{13}$$

The inequalities (11) now follow trivially from (12), (13) and the inclusion relations $S \subset K \subset \mathcal{P}$ and $\Theta \subset K \subset S$.

The bounds on the right of (9) and (10) can be written in the form $(\gamma_n n)^{n/n}$ where γ_n tends to a positive limit as n tends to infinity. It is easy to see that it is impossible to obtain such bounds with γ_n tending to zero as n tends to infinity. The bounds in (11) are considerably closer than those obtained by K. Mahler[7] but they are probably very far from the best possible.

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[2] *Théorie des Opérations Linéaires*, Warsaw, 1932, p. 240.

[3] E.g., Orlicz, W., *Stud. Math.*, **4**, 51–47 (1933); Macphail, M. S., *Bull. Am. Math. Soc.*, **53**, 121–123 (1947).

[4] *Duke Math. J.*, **13**, 351–365 (1946).

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[6] I.e., the closure of a bounded open set.

[7] *Časopis Pěst. Mat. Fys.*, **68**, 93–102 (1939).

THE REGION OF VALUES OF THE DERIVATIVE OF A SCHLICHT FUNCTION*

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1. We say that a function

$$f(z) = \sum_{\nu=1}^{\infty} a_\nu z^\nu$$

belongs to class $\mathfrak{S}$ if it is regular and schlicht in $|z| < 1$ and $a_1 = 1$. The purpose of this note is to indicate how the variational methods developed by A. C. Schaeffer and D. C. Spencer[1, 2] can be used to determine the region of values covered by $f'(z_0)$, when $f(z)$ ranges over the class $\mathfrak{S}$, and z_0 is any fixed point in $|z| < 1$. A more detailed discussion will appear in reference 2. We denote the region by $R(z_0)$. Henceforth, without explicit statement to the contrary, $f(z)$ belongs to class $\mathfrak{S}$.

The region $R(z_0)$ is essentially the solution to a more general problem. Let

$$f(w) = w + a_2 w^2 + a_3 w^3 + \ldots$$

be regular and schlicht in a simply connected domain $\mathfrak{D}$ containing the origin, and let $w = w(z)$ be the function belonging to class $\mathfrak{S}$ which maps the unit circle on $\mathfrak{D}$. If we write $f(w) = F(z)$, then $F(z)$ belongs to class $\mathfrak{S}$. Writing $w_0 = w(z_0)$, we have $F'(z_0) = f'(w_0)w'(z_0)$. Since $w'(z_0)$ depends only on $\mathfrak{D}$ and z_0, not on f, it follows that the region of values at the point w_0 of functions regular and schlicht in $\mathfrak{D}$ is simply a Euclidean magnification and rotation of $R(z_0)$, the magnification factor being $z'(w_0)$.

It suffices to consider the case where z_0 is real and positive, because $F(z) = e^{-i\theta}f(e^{i\theta}z)$ belongs to class $\mathfrak{S}$, and $F'(z) = f'(e^{i\theta}z)$. We shall therefore take $z_0 = r$, $0 \le r < 1$. Before explicitly determining $R(r)$, various of its geometrical properties are readily established.

Since the class $\mathfrak{S}$ is compact, $R(r)$ is closed. It is also bounded, the precise bounds being given by the well-known distortion and rotation theorems. $R(r)$ has two symmetries. It is symmetric with respect to the real axis, since $\overline{f(\bar{z})}$ belongs to class $\mathfrak{S}$. Making the transformation $z = (w + r)/(1 + rw)$ and then normalizing, $f(z)$ is transformed into a function $F(w)$, belonging to class $\mathfrak{S}$, for which

$$F'(-r)f'(r) = 1/(1 - r^2)^2.$$

Thus $R(r)$ is symmetric by inversion in the circle about the origin of radius $1/(1 - r^2)$.

Clearly $R(r)$ varies continuously with r, for if $f'(r)$ is any point of $R(r)$, then given ϵ, there exists a δ such that $\left|f'(r + \Delta r) - f'(r)\right| < \epsilon$ for $\left|\Delta r\right| < \delta$, so there is a point of $R(r + \Delta r)$ arbitrarily near any point of $R(r)$ for Δr sufficiently small. The region $R(r)$ expands with increasing r; that is, $R(r_1) \subset R(r_2)$ for $r_1 < r_2$. Writing $r_1 = \rho r_2$, $0 < \rho < 1$, let $g(z) = f(\rho z)/\rho$. Then $g(z)$ belongs to class $\mathfrak{S}$, and $g'(r_2) = f'(r_1)$. Thus any point $f'(r_1)$ of $R(r_1)$ is a point $g'(r_2)$ of $R(r_2)$.

If $f'(r)$ is an interior point of $R(r)$, then $f'(r)$ belongs to $R(r - \Delta r)$ provided that Δr is sufficiently small. Hence there is a function $g(z)$ of class $\mathfrak{S}$ such that $g'(r - \Delta r) = f'(r)$; that is, $g'(\rho r) = f'(r)$, where $\rho = (r - \Delta r)/r$. Writing $h(z) = g(\rho z)/\rho$, we have $h'(r) = f'(r)$, where $h(z)$ is bounded in $\left|z\right| < 1$. Conversely, if a function $f(z)$, belonging to the point $f'(r)$ of $R(r)$, is bounded or satisfies the weaker condition that the map of $\left|z\right| < 1$ by $w = f(z)$ has an exterior point w_0, then we can find an $\epsilon > 0$ such that the circle $\left|w - w_0\right| < \epsilon$ is exterior to the map of the unit circle by $f(z)$. It follows that the function

$$g(z) = \frac{w_0{}^2}{w_0{}^2 - e^{2i\theta}\epsilon^2} \left[f(z) + \frac{e^{2i\theta}\epsilon^2}{f(z) - w_0} \right] + \frac{w_0 e^{2i\theta}\epsilon^2}{w_0{}^2 - e^{2i\theta}\epsilon^2}$$

belongs to class $\mathfrak{S}$, where θ is an arbitrary real number. Differentiating, we find that

$$g'(r) = \left\{ 1 + \frac{f(r)[f(r) - 2w_0]}{w_0{}^2[f(r) - w_0]^2} e^{2i\theta}\epsilon^2 + o(\epsilon^2) \right\} f'(r),$$

which implies that a complete neighborhood of $f'(r)$ belongs to $R(r)$, and $f'(r)$ is an interior point.

If $0 \leq \rho < 1$, the function $f(\rho z)/\rho$ belongs to the point $f'(\rho r)$ of $R(r)$. The point $f'(\rho r)$ is an interior point of $R(r)$, since $f(\rho z)/\rho$ is bounded, and it follows that $R(r)$ is a closed domain. We note that if θ is real, then the curve $f'(re^{i\theta})$ lies in $R(r)$. Hence at a point $f'(r)$ on the boundary of $R(r)$ where it has a tangent, $f''(r)$ defines a vector normal to the boundary. It will be shown later that the boundary of $R(r)$ has a tangent everywhere.

Although it will also be shown in the sequel that $R(r)$ is simply connected, the region is not schlicht for $r > 1/\sqrt{2}$. This is owing to the fact that $\arg f'(r)$, $\arg f'(0) = 0$, can exceed π in magnitude when $r > 1/\sqrt{2}$ For this reason it is better to consider the set of values at $z = r$ of that branch of $\log f'(z)$ which vanishes at $z = 0$. Since for any function $f(z)$ the derivative $f'(z)$ does not vanish in $\left|z\right| < 1$, we see that any branch of $\log f'(z)$ is single-valued in $\left|z\right| < 1$. Let $L(r)$ be the domain of values of the branch of $\log f'(z)$ at $z = r$ which vanishes at $z = 0$.

For convenience we refer to functions $f(z)$ for which $\log f'(r)$ lies on the boundary of $L(r)$ as boundary functions. To each point on the boundary

there corresponds a unique boundary function. Henceforth, without explicit statement to the contrary, we will interpret log $f'(z)$ as that branch defined in $|z| < 1$ which vanishes at $z = 0$.

2. It was proved by Schaeffer and Spencer[3] that boundary functions must satisfy the differential equation

$$-\alpha e^{i(\varphi + \psi)}\left(\frac{z}{w}\frac{dw}{dz}\right)^2 \frac{2w - \alpha}{(w - \alpha)^2} =$$
$$e^{-i(\varphi + \psi)}\frac{(z - e^{i\psi})^2(z - \rho e^{i\varphi})(z - e^{i\varphi}/\rho)}{(z - r)^2(z - 1/r)^2}, \qquad (2.1)$$

where the right side is non-negative on $|z| = 1$, and

$$-e^{2i(\varphi + \psi)}(1 - r^2)^2 = (r - e^{i\psi})^2(r - \rho e^{i\varphi})(r - e^{i\varphi}/\rho) \qquad (2.2)$$

Here φ, ψ and ρ are real constants, $0 < \rho \leq 1$, and $\alpha = w(r)$. Integrating (2.1) and taking limits as $z \to 0$ and $z \to r$, we obtain

$$\log f'(r) = 2 \log \frac{1 - re^{-i\psi}}{1 - r^2} + 2i \log \frac{s(r) + \rho}{s(r) - \rho}$$
$$+ e^{-i(\varphi + \psi)}\left\{\log \frac{(1 - \rho)(\rho - |s(r)|^2)}{(1 + \rho)(\rho + |s(r)|^2)} + 2 \arg \frac{1 + s(r)}{1 - s(r)}\right\} + \pi, \quad (2.3)$$

where

$$s^2(z) = \frac{z - \rho e^{i\varphi}}{z - e^{i\varphi}/\rho}$$

The logarithms and argument are principal values, and $Im\ s(r) < 0$. An indeterminacy of $2m\pi + 2n\pi i$ has been resolved through the substitution of the Koebe functions, $f(z) = z/(1 \pm z)^2$, which are known to be boundary functions.

For $0 < r \leq 1/\sqrt{2}$, the region $L(r)$ is bounded by a closed curve given by (2.3). This is actually the equation of a curve, since there is but one real independent variable; indeed, for each ψ there are unique ρ and $e^{i\varphi}$ which satisfy (2.2). Any boundary function $w = f(z)$ maps $|z| < 1$ onto the exterior of a single analytic slit in the w-plane extending to $w = \infty$.

For $1/\sqrt{2} < r < 1$, the region $L(r)$ is bounded by two arcs alternating with two straight-line segments. The arcs are given by (2.3) for ψ in the intervals

$$-\pi/4 + \tau < \psi < \pi/4 - \tau,$$
$$\pi/4 + \tau < \psi < 3\pi/4 - \tau. \qquad (2.4)$$

Here $\tau = \tau(r) = \arccos 1/\sqrt{2}r$, $0 < \tau < \pi/4$. A boundary function corresponding to a point on these arcs maps $|z| < 1$ onto the exterior of a single analytic slit in the w-plane extending to $w = \infty$ as in the preceding

case. For ψ at the ends of the intervals (2.4), we obtain the remainder of the boundary of $L(r)$, consisting of the two straight-line segments of extreme imaginary part

$$C_0(r) \leq Re \log f'(r) \leq C_1(r),$$

$$Im \log f'(r) = \pm (\log \frac{r^2}{1 - r^2} + \pi), \tag{2.5}$$

where

$$C_0(r) = -\log (r^2 + \sqrt{2r^2 - 1}) + 2r,$$

$$C_1(r) = -\log (r^2 - \sqrt{2r^2 - 1}) - 2r.$$

A boundary function corresponding to a point on these line segments has extreme imaginary part and maps $|z| < 1$ onto the exterior of a slit in the w-plane. The slit consists of a straight-line segment extending from a finite point to $w = \infty$ and two arcs which are symmetrical with respect to the straight-line segment and meet it at its finite end to form three equal angles. In the case of boundary functions corresponding to the end-points of the line segments (2.5), one of the two arcs degenerates into a point.

3. For fixed r, the boundary of $R(r)$ has been represented in parametric form, the boundary functions varying continuously with the parameter of the boundary. If $f(z)$ is a boundary function, then $F(z) = f(\rho z)/\rho$ belongs to class $\mathcal{S}$ for $0 \leq \rho \leq 1$. Letting ρ vary from 1 to 0, we obtain a homotopic deformation of the boundary of $R(r)$ into the point $F'(r) = 1$. Thus $R(r)$ is simply connected.

For $0 < r \leq 1/\sqrt{2}$ the boundary of $R(r)$ has been shown to be analytic, and for $1/\sqrt{2} < r < 1$, it has been shown to be analytic with the exception of four points. We will now show that even at these four points the boundary has a tangent. Consider the function

$$\Phi(z) = f \left(\frac{z + \eta}{1 + \bar{\eta} z} \right),$$

where η is a small complex number. The function

$$F(z) = \frac{1}{\Phi'(0)} [\Phi(z) - \Phi(0)]$$

clearly belongs to class $\mathcal{S}$. Now let $f(z)$ extremalize $Im[e^{-i\tau}f'(r)]$, where τ is some fixed real number. Upon differentiating $Im[e^{-i\tau}F'(r)]$ with respect to η, it readily follows that

$$e^{-i\tau}[f''(r) - 2a_2 f'(r)] + e^{i\tau} r[r\overline{f''(r)} + 2\overline{f'(r)}] = 0. \tag{3.1}$$

Let $f(s)$ be the boundary function corresponding to one of the points where the straight-line segments (2.5) meet the arcs (2.3). If $f''(r) \neq 0$, the curve $f'(re^{i\theta})$, θ real, lies in $R(r)$ and has a tangent at the point in question. Hence the interior angle of the boundary at the point is not less than π. If $f''(r) = 0$, then by (3.1)

$$a_2 e^{-ir} f'(r) = re^{ir} \overline{f'(r)}. \tag{3.2}$$

Since $f'(r) \neq 0$, we see that $a_2 \neq 0$. If the internal angle were less than π, there would be infinitely many values of r satisfying (3.2) for the same function $f(s)$, and so for the same values of a_2 and $f'(r)$. This is impossible. Hence the internal angle at the point is not less than π. However, the internal angle cannot exceed π, since $\arg f'(r)$ is maximal at the point. Therefore the internal angle is precisely equal to π.

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[1] Schaeffer, A. C., and Spencer, D. C., "A Variational Method in Conformal Mapping," *Duke Math. J.*, 14, 949–966 (1947).

[2] Schaeffer, A. C., and Spencer, D. C., "Coefficient Regions for Schlicht Functions," to appear as an *American Mathematical Society Colloquium Publication*.

[3] Schaeffer, A. C., and Spencer, D. C., "A General Class of Problems in Conformal Mapping," these Proceedings, 33, 185–189 (1947).

A GENERALIZATION OF POISSON'S DISTRIBUTION FOR MARKOFF CHAINS*

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1. *Introduction.*—Let $U_{n,k}$ ($n = 1, 2, \ldots$; $k = 1, \ldots, n$) be an infinite triangular array of variates (chance variables). If each row ($U_{n,1}, \ldots, U_{n,n}$) represents a set of n independent trials (i.e., $U_{n,k} = 1$ or 0 according as the kth trial succeeds or fails), and if $P_n(s)$ is the probability of exactly s successes in this set, the Poisson distribution of mean m, $P_n(s) \rightarrow P(s) = e^{-m} m^s / s!$, is obtained in the case in which the individual probabilities of success, $p_{n,k} = p(U_{n,k} = 1)$, become small while the expected total number of successes approach m, as the number n of trials increases indefinitely (we are using $p(A)$ and $p(A|B)$ for the probability of event A, and the probability of A given B; and E will be used for the expected value). More precisely, the equations

$$\lim_{n \to \infty} \max_{1 \leq k \leq n} p_{n,k} = 0, \tag{1.1}$$

$$\lim_{n \to \infty} E\left(\sum_{k=1}^{n} U_{n, k}\right) = \lim_{n \to \infty} \sum_{k=1}^{n} p_{n, k} = m \geq 0 \qquad (1.2)$$

together form the necessary and sufficient condition that $P_n(s)$ approach the Poisson distribution.[1] The latter is also called the *Law of Small Numbers*.

The present note considers the case in which each set of trials $(U_{n, 1}, \ldots, U_{n, n})$, instead of being independent, forms a simple Markoff chain of length n.

In this case, in addition to the absolute probabilities $p_{n, k}$, the transition probabilities $(k = 2, \ldots, n)$:

$$\left.\begin{array}{ll} a_{n, k} = p(U_{n, k} = 1 | U_{n, k-1} = 1), & a'_{n, k} = 1 - a_{n, k}, \\ b_{n, k} = p(U_{n, k} = 1 | U_{n, k-1} = 0), & b'_{n, k} = 1 - b_{n, k}, \end{array}\right\} \quad (1.3)$$

play an essential role. They are connected by the classical difference equation $(k = 2, \ldots, n)$:

$$p_{n, k} = r_{n, k}\, p_{n, k-1} + b_{n, k}, \qquad (r_{n, k} = a_{n, k} - b_{n, k}). \qquad (1.4)$$

To seek a generalization of Poisson's distribution for this case, we might impose condition (1.1). But the following more liberal condition:

$$\lim_{n \to \infty} p_{n, 1} = 0 \; implies \; \lim_{n \to \infty} \max_{1 \leq k \leq n} p_{n, k} = 0 \qquad \cdot (1.5)$$

would seem to be more appropriate. It corresponds to the view that the essential features of the mechanism by means of which the trials are made are expressed by its transition probabilities, whereas the absolute probabilities are more incidental and are largely determined by the situation in the first trial.

In the Markoff case (implying (1.4) or its generalization), we will say that any limiting distribution $P_n(s) \to P(s)$ obtained under conditions implying (1.2) and (1.5) is a *law of small numbers*.

We shall establish a law of small numbers in the case of stationary transitions $(a_{n, k}, b_{n, k}$ independent of k); its generating function is of a simple exponential form and the probabilities $P(s)$ are given in terms of Laguerre polynomials. The note concludes with a statement, without proof, of the theorem giving the distribution in the non-stationary case, and considers further generalizations.

2. *Preliminaries on Markoff Chains.*—Let $\sigma_{n, k}$ be the standard deviation of $U_{n, k}$, and $\rho_{n, j, k}$ the correlation coefficient of $U_{n, j}, U_{n, k}$ $(1 \leq j \leq k \leq n)$. Then it is easily seen from the difference equations of type (1.4) that[2] $\rho_{n, j, k} = r_{n, j+1}^{(k-j)} \sigma_{n, j}/\sigma_{n, k}$. Therefore the coefficient of regression of $U_{n, k}$ on $U_{n, j}$, being $\rho_{n, j, k}\, \sigma_{n, k}/\sigma_{n, j}$, is $r_{n, j+1}^{(k-j)}$.

To find the generating function of $P_s(s)$,

$$\varphi_n(t) = \sum_{s=0}^{n} P_s(s)t^s, \tag{2.1}$$

we introduce $u_{n,\,k,\,s}$, the probability that there be exactly s successes in the trials $(U_{n,\,1}, \ldots, U_{n,\,k})$ $(0 \leq s \leq k \leq n)$. If $u'_{n,\,k,\,s}$ is the probability of this event and also that success occur on the kth trial and if $u''_{n,\,k,\,s}$ is a similar probability with failure in the kth, we have

$$u_{n,\,k,\,s} = u'_{n,\,k,\,s} + u''_{n,\,k,\,s}, \qquad u'_{n,\,k,\,0} = 0, \qquad u''_{n,\,k,\,k} = 0.$$

Simple probability reasoning establishes the classical difference equations

$$u'_{n,\,k+1,\,s+1} = a_{n,\,k+1}\, u'_{n,\,k,\,s} + b_{n,\,k+1}\, u''_{n,\,k,\,s}$$

$$u''_{n,\,k+1,\,s} = a'_{n,\,k+1}\, u'_{n,\,k,s} + b'_{n,\,k+1}\, u''_{n,\,k,\,s}.$$

Introducing the generating functions

$$\varphi_{n,\,k}(t) = \sum_{s=0}^{k} u_{n,\,k,\,s}\, t^s, \qquad \varphi'_{n,\,k}(t) = \sum_{s=0}^{k} u'_{n,\,k,\,s}\, t^s,$$

$$\varphi''_{n,\,k}(t) = \sum_{s=0}^{k} u''_{n,\,k,\,s}\, t^s,$$

we evidently have

$$\varphi_{n,\,k}(t) = \varphi'_{n,\,k}(t) + \varphi''_{n,\,k}(t), \qquad \varphi_{n,\,n}(t) = \varphi_n(t).$$

Furthermore, the above difference equations lead at once to the following matrix recurrence equation

$$\Phi_{n,\,k+1} = \Phi_{n,\,k} W_{n,\,k+1} \qquad (1 \leq k \leq n-1),$$

where

$$\Phi_{n,\,k} = \|\varphi'_{n,\,k}(t)\ \varphi''_{n,\,k}(t)\|,$$

$$W_{n,\,k} = \left\|\begin{matrix} ta_{n,\,k} & a'_{n,\,k} \\ tb_{n,\,k} & b'_{n,\,k} \end{matrix}\right\|.$$

Combining these relations, we obtain the expression

$$\varphi_n(t) = \|tp_{n,\,1}\ q_{n,\,1}\| W_{n,\,2} W_{n,\,3} \ldots W_{n,\,n} J, \qquad J = \left\|\begin{matrix} 1 \\ 1 \end{matrix}\right\|.$$

3. The Case of Stationary Transitions.—In this section we assume that the transition probabilities $a_{n,\,k}$, $b_{n,\,k}$ are *stationary*, implying the existence of the infinite sequences $\{a_i\}$, $\{b_i\}$, with

$$a_{n,\,k} = a_n, \qquad b_{n,\,k} = b_n, \qquad (a'_n = 1 - a_n, \qquad b'_n = 1 - b_n). \tag{3.1}$$

The condition (1.5) applied to (1.4) leads to $b_n \to 0$. We assume further that

$$\lim_{n \to \infty} a_n = a < 1, \qquad \lim_{n \to \infty} p_{n, 1} = p_1, \qquad (q_1 = 1 - p_1). \quad (3.2)$$

Summing (1.4) for $k = 2, \ldots, n$, and applying these relations, we obtain (since $a = \lim r_n = r$)

$$\left. \begin{array}{l} b_n = h/n + \theta_n/n, \\ h = m(1 - r) - p_1, \qquad \lim_{n \to \infty} \theta_n = 0. \end{array} \right\} \quad (3.3)$$

Since $a < 1$ we may fix $r > 1$ with $ar < 1$. Let T be the circle $|t| \leq r$ in the complex t plane. We will show that $\varphi_n(t) \to \varphi(t)$ uniformly on T as $n \to \infty$. From this it will follow, by use of Cauchy's integral formulas for the coefficients of t^s in $\varphi_n(t)$ and $\varphi(t)$ as contour integrals about the circle $|t| = r$, that $P_n(s) \to P(s)$, generated by $\varphi(t)$.

Applying the formulas of this section to the results at the end of section 2, we see that, once the uniform limit on T of $W_{n, 2}^{(n - 1)} = W_n^{n - 1}$ has been found, we shall have, uniformly on T,

$$\varphi_n(t) \to \varphi(t) = \|tp_1 \, q_1\| WJ, \qquad W_n^{n - 1} \to W. \quad (3.4)$$

To find W, reduce W_n to diagonal form. Its characteristic roots are

$$\left. \begin{array}{l} \lambda'_n \\ \lambda''_n \end{array} \right\} = \frac{1}{2} \left\{ b'_n + ta_n \mp [(b'_n + ta_n)^2 - 4t \, r_n]^{1/2} \right\}; \quad (3.5)$$

and we have, in virtue of (3.1)–(3.3),

$$\lim_{n \to \infty} \lambda'_n = ta, \qquad \lim_{n \to \infty} \lambda''_n = 1. \quad (3.6)$$

Since for all $t \, \epsilon \, T$, $|ta| < 1$, these roots are distinct once n is sufficiently large. Then $W_n = K_n \Lambda K_n^{-1}$, where

$$\Lambda_n = \left\| \begin{array}{cc} \lambda'_n & 0 \\ 0 & \lambda''_n \end{array} \right\|, \qquad K_n = \left\| \begin{array}{cc} a'_n & a''_n \\ \lambda'_n - ta_n & \lambda''_n - ta_n \end{array} \right\|. \quad (3.7)$$

The limits of K_n, K_n^{-1}, being uniform on T, we have but to find the uniform limit $\Lambda_n^{n - 1} \to \Lambda$, obtaining

$$W = \left\| \begin{array}{cc} a' & a' \\ 0 & 1 - at \end{array} \right\| \Lambda \left\| \begin{array}{cc} 1/a' & -1/(1 - at) \\ 0 & 1/(1 - at) \end{array} \right\|. \quad (3.8)$$

In view of (3.6) and (3.7),

$$\Lambda = \lim_{n \to \infty} \left\| \begin{array}{cc} (\lambda'_n)^{n-1} & 0 \\ 0 & (\lambda''_n)^{n-1} \end{array} \right\| = \left\| \begin{array}{cc} 0 & 0 \\ 0 & \lambda \end{array} \right\|, \quad (3.9)$$

where $\lambda = \lim (\lambda''_n)^{n-1} = \lim (\lambda''_n)^n$ (all limits uniform on T)

Direct integration shows that

$$(1 + z)^{1/2} = 1 + \frac{z}{2} - \frac{z^2}{4} \int_0^1 \frac{(1 - v)\, dv}{(1 + vz)^{3/2}}, \qquad (|z| < 1),$$

the path of integration being the real segment $(0, 1)$. Applying this to (3.5), and making use of (3.2) and (3.3), we find that

$$\lambda_n'' = 1 - \frac{h(1 - t)}{1 - rt} \frac{1}{n} + \frac{\Omega_n(t)}{n}, \tag{3.10}$$

where $\Omega_n(t) \to 0$ uniformly on T as $n \to \infty$.

Integration along the real segment $(0 \le v \le 1)$ shows that

$$\log (1 + z) = z - z^2 \int_0^1 \frac{(1 + v)\, dv}{(1 + vz)^2}, \qquad (|z| < 1).$$

Applying this and (3.10) to $\log (\lambda''_n)^n$, we finally derive the limit, uniform on T:

$$(\lambda''_n)^n \to \lambda = \exp\left[- \frac{h(1 - t)}{1 - rt} \right]. \tag{3.11}$$

Combining (3.3), (3.4) (3.8), (3.9), (3.11), we have:

THEOREM I. *If a Markoff chain with stationary transitions satisfies the small number conditions* (1.2), (1.5), *and the limit conditions* (3.2), *then it leads to a law of small numbers* $P_n(s) \to P(s)$, *generated by*

$$\varphi(t) = \left[1 - \frac{(1 - t)p_1}{1 - rt} \right] \exp \frac{-m\,(1 - r)(1 - t) + (1 - t)p_1}{1 - rt}. \tag{3.12}$$

Explicit expressions for $P(s)$ are easily found in terms of the Laguerre polynomials, defined by the equation

$$\frac{1}{1 - x} \exp \frac{-wx}{1 - x} = \sum_{s=0}^{\infty} L_s(w)x^s.$$

On setting

$$x = rt, \qquad w = -[m(1 - r) - p_1]\,(1 - r)/r,$$

we obtain

$$P(s) = e^{-m(1-r)+p_1} [q_1 L_s(w)r^s + (p_1 - r)L_{s-1}(w)r^{s-1}].$$

4. *The Case of Non-Stationary Transitions.*—We continue to assume the small number condition (1.5); but (1.2) must be extended to take the form that, for each $v = 0, 1, 2, \ldots,$

$$\lim_{n \to \infty} \sum_{k=1}^{n-v} E(U_{n,\,k} U_{n,\,k+v}) = R_v. \tag{4.1}$$

Evidently $R_i = m$. Furthermore (3.1) and (3.2) are replaced by

$$a_{n,k} \to a_k \leqq a < 1 \ uniformly \ on \ (2 \leqq k \leqq n) \ as \ n \to \infty; \quad (4.2)$$

$$b_{n,k} \left[\sum_{k=2}^{n} b_{n,k} \right]^{-1} = c_k \left[\sum_{k=2}^{\infty} c_k \right]^{-1} (1 + \epsilon_{n,k}), \quad (4.3)$$

where $\epsilon_{n,k} \to 0$ uniformly as $n \to \infty$: (4.3) is a statement of the uniform approach of $b_{n,1} : b_{n,2} : \ldots$ to limiting proportions $c_2 : c_3 : \ldots$ (c_i not all zero). We then have

THEOREM II. *Under hypotheses (1.5), (4.1), (4.2), (4.3), and the assumption $p_{n,1} \to p_1$, there follows the conclusion that $P_n(s) \to P(s)$ generated by the function*

$$\varphi(t) = [1 - f(t)] \exp \{ -(1-t)[m + (t-1)\psi(t)] + f(t) \}, \quad (4.4)$$

where

$$\psi(t) = \sum_{\nu=1}^{\infty} R_\nu t^{\nu-1}, \quad f(t) = (1-t) \sum_{\nu=0}^{\infty} p_1 a_2^{(\nu)} t^\nu. \quad (4.5)$$

The proof depends on a certain theorem in analysis.[3] Similar methods have recently led the author to formally similar results in the case where each trial has many possible outcomes (e.g., corresponding to points on an N-space), one class of which corresponds to "success," the rest to "failure"; matrix and operator multiplication enter.

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[*] An outline of the paper which will appear in full under the title, "A Law of Small Numbers in Markoff Chains," presented to the American Mathematical Society in its meeting in New York, February 26, 1949. The investigation was suggested by the theoretical (and unclassified) part of developements made in 1948 by the author in the course of his work with the Operations Evaluation Group of the Chief of Naval Operations, U. S. Navy. Any views contained in this work are the author's and not the Navy's.

[1] Koopman, B. O., "Necessary and Sufficient Conditions for Poisson's Distribution," *Bull. Am. Math. Soc.* (forthcoming).

[2] We shall agree to write
$A_{n,i}^{(0)} = 1, \quad A_{n,i}^{(\lambda)} = A_{n,i} A_{n,i+1} \ldots A_{n,i+\lambda-1},$
whatever the sequence of symbols $A_{n,i}$ ($i = 1, 2, \ldots$) may denote. In the present case, $r_{n,i}^{(k, -, i)} = r_{n,i+1} r_{n,i+2} \ldots r_{n,k}$.

[3] Cf. Koopman, B. O., "Exponential Limiting Products in Banach Algebras" (forthcoming).

ALMOST PERIODIC INVARIANT VECTOR SETS IN A METRIC VECTOR SPACE

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In this note it will be shown that the axioms on which H. Weyl, in a recent paper published under the above title,[1] has based his theory may be reduced by a somewhat different approach. In particular, we shall be able to dispose of the axiom IV the artificial character of which he himself deplores.

We start from the main theorem of the theory of almost periodic functions in a group (see reference 2), but we prefer to express it in this way: Given a group $\mathfrak{G}$ and a closed modul $\mathfrak{M}$ of almost periodic functions in $\mathfrak{G}$. If $\mathfrak{M}$ is invariant under the transformations $f(x) \rightarrow f(cx)$, c arbitrary in $\mathfrak{G}$, we call $\mathfrak{M}$ a left-modul. Then we have: *Given an arbitrary left-modul $\mathfrak{M}$ consisting of almost periodic functions, this modul is always the smallest closed modul, containing all (finite) irreducible left-moduls in $\mathfrak{M}$.* (See reference 4.)

A theorem like this is correct also for abstract almost periodic functions in $\mathfrak{G}$. A function $f(x)$ on a group is called an abstract function, if the values f of the function are elements of a vector space $\mathfrak{B}$ instead of complex numbers. The abstract vector space $\mathfrak{B}$ (possibly with non-denumerable dimensions) is a linear space with the complex numbers as left-operators. (If we assume that the elements of $\mathfrak{G}$ are right-operators, we have a vector space, satisfying the axiom I in reference 1.) Besides this we suppose the elements $u \, \epsilon \, \mathfrak{B}$ to have a length $|u|$. We require $\mathfrak{B}$ to be closed in the sense of this length. [We see, that $\mathfrak{B}$ satisfies the axiom III in reference 1, but without the formula (1.3). Besides this we do not assume the invariance of $|u|$, because the transformation with elements of the group is not necessarily defined.] The notion "almost periodic function with values in $\mathfrak{B}$" is defined in the same manner as in the case where $\mathfrak{B}$ consists of complex numbers (compare reference 5). The word "left-modul," also, is to have the same meaning as before, properly adjusted to the new case. From the main theorem, given above, and from the fact, that every abstract almost periodic function $f(x)$ can be uniformly approximated with arbitrary accuracy by folded functions $\varphi \times f$ with complex almost periodic functions φ, we conclude the *main-theorem* about abstract almost periodic functions: *Given an arbitrary left-modul $\mathfrak{M}$, consisting of abstract almost periodic functions, this modul $\mathfrak{M}$ is always the smallest closed modul, containing all (finite) irreducible left-moduls in $\mathfrak{M}$.*

From now on we suppose, that $\mathfrak{B}$ has the elements of $\mathfrak{G}$ as right-operators and that the length $|u|$ is invariant under the transformations of $\mathfrak{G}$. [We see, that $\mathfrak{B}$ fulfills the axioms I and III of reference 1, but without the inequality (1, 3).] Then a vector $u \in \mathfrak{B}$ is called almost periodic (as in reference 1), if $f(x) = ux$ is an almost periodic function of x. Now we consider a closed, invariant modul $\mathfrak{U} \subseteq \mathfrak{B}$, consisting of almost periodic vectors $u \in \mathfrak{B}$ only. Then the set of all functions $f(x) = ux$ with $u \in \mathfrak{U}$ is a left-modul of almost periodic functions. If we apply the above-mentioned main-theorem concerning abstract almost periodic functions, we obtain the *approximation theorem: Given an arbitrary invariant closed modul $\mathfrak{U}$, consisting of almost periodic vectors $\in \mathfrak{B}$, this modul $\mathfrak{U}$ is always the smallest closed modul, containing all (finite) irreducible moduls in $\mathfrak{B}$.* This theorem is more general than Weyl's "theorem of strong approximation,"[1] which can be deduced from our theorem by mere specialization.

The Parseval equation is an immediate consequence of our approximation theorem. But now it is necessary to have a scalar product (u, v) of the vectors $u, v \in \mathfrak{B}$, which is invariant under the transformations of $\mathfrak{G}$. Moreover it must be assumed that $\sqrt{(u, u)} = \|u\| \leq |u|$. (This is the inequality (1.3) of the axiom III in reference 1. From now on we suppose that all assumptions of Weyl's axioms I, II and III are fulfilled.) $\mathfrak{U} \subseteq \mathfrak{B}$ being a closed invariant modul, consisting of almost periodic vectors, we examine all (finite) invariant irreducible moduls $\mathfrak{U}' \subseteq \mathfrak{U}$. Each gives rise to an irreducible representation $D'(x)$ of $\mathfrak{G}$. All $\mathfrak{U}'$ belonging in this sense to a representation $D'(x)$ equivalent to a fixed representation $D(x)$, we unite into a modul $\mathfrak{U}_D$. We suppose, that all moduls $\mathfrak{U}_D$ are of finite dimension. (Denumerable dimensions would be also admissible. The invariant sets, which are considered by Weyl, are of this kind.) Then we can find in every $\mathfrak{U}_D$ an orthogonal and normed basis $e_1^{(D)}, \ldots, e_{n_D}^{(D)}$. If now D runs over a complete, not necessarily denumerable, system of inequivalent irreducible representations $\ldots, D^{(\nu)}(x), D^{(\mu)}(x), \ldots$ of $\mathfrak{G}$ we obtain an orthogonal and normed system of vectors $\ldots, e_1^{(\nu)}, \ldots,$ $e_{n_\nu}^{(\nu)}, e^{(\mu)}, \ldots, e_{n_\mu}^{(\mu)}, \ldots$, in $\mathfrak{U}$. Making use of $\|u\| \leq |u|$, we deduce from the approximation theorem, that every vector $u \in \mathfrak{U}$ can be approximated in the mean (that is, in the sense of $\|u\|$) with arbitrary accuracy by finite linear combinations of the $e_i^{(\nu)}$. For because of the approximation theorem, this is correct even in the strong case (that is, in the sense of $|u|$). In the usual manner one concludes from this the *Parseval equation: We define the Fourier-coefficients of an almost periodic vector $u \in \mathfrak{U}$ to be the numbers $\alpha_i^{(\nu)} = (u, e_i^{(\nu)})$. Then we have*

$$\|u\|^2 = \sum_\nu \sum_i |\alpha_i^{(\nu)}|^2.$$

A fuller treatment of the theory outlined above will appear in *Mathematische Annalen*.

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ON MEMBRANES AND PLATES

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I. Introduction.—1. We deal with three closely related problems of the Calculus of Variations having some connection with the theory of elastic deformations.

Let D be an arbitrary domain in the plane bounded by a simple curve C. We denote the area-element of D by $d\sigma$. The admissible functions u are defined in D and satisfy certain boundary conditions on C. We define the positive numbers $\lambda_1, \lambda_2, \lambda_3$ as follows:

$$\lambda_1^2 = \min. \frac{\int_D \int |\operatorname{grad} u|^2 \, d\sigma}{\int_D \int u^2 \, d\sigma}, \qquad u = 0 \text{ on } C, \qquad (a)$$

$$\lambda_2^4 = \min. \frac{\int_D \int (\nabla^2 u)^2 \, d\sigma}{\int_D \int u^2 \, d\sigma}, \qquad u = \frac{\partial u}{\partial n} = 0 \text{ on } C, \qquad (b)$$

$$\lambda_3^2 = \min. \frac{\int_D \int (\nabla^2 u)^2 \, d\sigma}{\int_D \int |\operatorname{grad} u|^2 \, d\sigma}, \qquad u = \frac{\partial u}{\partial n} = 0 \text{ on } C. \qquad (c)$$

Obviously, $\lambda_2^2 \geqq \lambda_1 \lambda_3$.

In (a) we allow only continuous functions which have piecewise continuous first derivatives. In (b) and (c) we allow only functions with continuous first derivatives. For the sake of simplicity we assume that C is an analytic curve.

2. Problems (a) and (b) are classical; the quantities λ_1 and λ_2 are the fundamental frequencies of a membrane with fixed boundary and of a clamped plate, respectively. Problem (c) occurs in the study of the buckling of plates.[1] All the three problems have a considerable literature for which we refer to the book of Weinstein.

As to (a) Lord Rayleigh has formulated the following conjecture which

was first proved by G. Faber:[2] *For all membranes of given area the circular one has the gravest fundamental tone (lowest fundamental frequency).*

Our purpose is to prove an analogous theorem for the problems (b) and (c), i.e., for the quantities λ_2 and λ_3. This can be done under a certain hypothesis concerning the functions u for which the minima in (b) and (c) are attained. The *hypothesis* is the following: *The functions u minimizing problems (b) and (c) are different from zero throughout the domain D, that is, the fundamental vibrations do not produce any nodal lines.*

3. We note the Euler-Lagrange differential equations associated with the minimum problems formulated above:

$$\nabla^2 u + \lambda_1^2 u = 0, \tag{a'}$$

$$\nabla^2\nabla^2 u - \lambda_2^4 u = 0, \tag{b'}$$

$$\nabla^2\nabla^2 u + \lambda_3^2\nabla^2 u = 0. \tag{c'}$$

It is of interest to point out the minima in question for the special case of a circle. Denoting the radius of the circle by a we have

$$\lambda_1 = j/a, \qquad \lambda_2 = k/a, \qquad \lambda_3 = j'/a$$

where j, k, j' denote the smallest positive root of the Bessel functions

$$J_0(x), \qquad J_0(x)I_0'(x) - J_0'(x)I_0(x), \qquad J_0'(x),$$

respectively. We have

$$j = 2.405, \qquad k = 3.19, \qquad j' = 3.832. \tag{3}$$

Thus the principal result can be expressed as follows: *Let D be an arbitrary domain and let a be the radius of the circle of the same area as D. Then, under the hypothesis formulated above, we have the bounds*

$$\lambda_1 \geqq j/a, \qquad \lambda_2 \geqq k/a, \qquad \lambda_3 \geqq j'/a. \tag{4}$$

The first inequality is the content of the theorem of Rayleigh-Faber, the second and the third are proved in the present paper (under the hypothesis mentioned above). In all the three cases the proof is based on the process of symmetrization as it was in the proof of Faber. However we shall modify Faber's argument at a point which will be essential in dealing with the more difficult second and third problem.

II. Problem (a): Membrane with Fixed Boundary.—1. We denote by u the minimizing function of I (a) which is *known* to be different from zero in the interior of D. Hence, we can assume that $0 \leqq u \leqq 1$. We denote the level curve $u = \rho$ by C_ρ, $0 \leqq \rho \leqq 1$, so that $C_0 = C$ and C_1 coincides with the point(s) at which the maximum $u = 1$ is attained. The set C_ρ consists of a finite number of separated curves; for the sake of simplicity we assume that C_ρ is a single Jordan curve.

Let us denote by $A(\rho)$ the area of the domain inside of C_ρ. Thus $A(0) =$ A is the area of the given domain D, $A(1) = 0$. We define the quantity R by the equation $A(\rho) = \pi R^2$ so that R is a decreasing function of ρ; the maximum R_0 of R is the radius of the circle which has the same area as the given domain D.

2. We symmetrize the level curves C_ρ by replacing C_ρ by a circle of radius R about a fixed point, say the origin. The domain D is replaced then by a circular disk $\bar{D}$ of radius R_0. We define on $\bar{D}$ a function $\bar{u}$ by the condition that $\bar{u} = f(\rho)$ on the circle of radius R; the function f will be determined in such a way that the integral in the numerator of I (a) does not change in the transition from D to $\bar{D}$ and from u to $\bar{u}$. That is, if $\overline{d\sigma}$ is the area-element of $\bar{D}$,

$$\int_D \int |\,\mathrm{grad}\ u\,|^2\, d\sigma = \int_{\bar{D}} \int |\,\mathrm{grad}\ \bar{u}\,|^2\, \overline{d\sigma}. \tag{1}$$

On the other hand, we shall prove that

$$\int_D \int u^2\, d\sigma \leqq \int_{\bar{D}} \int \bar{u}^2\, \overline{d\sigma}. \tag{2}$$

Also the boundary condition $\bar{u} = 0$ will be satisfied. This yields the assertion immediately.

We observe that this argument differs from that of Faber. He defines the "symmetrized" function $\bar{u}$ by the condition $\bar{u} = \rho$ and shows that this process diminishes the integral in the numerator and leaves the integral in the denominator unchanged.

3. In what follows we use the notation

$$G = |\,\mathrm{grad}\ u\,| = \frac{d\rho}{dn} \tag{3}$$

where $d\rho > 0$ and dn is the piece of the normal of the level curve C_ρ between the level curves C_ρ and $C_{\rho+d\rho}$. The area of the ring-shaped domain between these curves is $A(\rho) - A(\rho + d\rho) = -A'(\rho)\, d\rho$. On the other hand, if ds is the arc-element of C_ρ, we find for the element $d\sigma$ of this area $d\sigma = ds \cdot dn$ $= ds \cdot d\rho/G$ so that

$$-A'(\rho) = |\,A'(\rho)\,| = \int_{u\,=\,\rho} G^{-1}\, ds. \tag{4}$$

By Schwarz's inequality

$$\int_{u\,=\,\rho} G\, ds \int_{u\,=\,\rho} G^{-1}\, ds \geqq \left(\int_{u\,=\,\rho} ds\right)^2 = (L(\rho))^2 \tag{5}$$

where $L(\rho)$ is the length of C_ρ. Using the isoperimetric inequality $(L(\rho))^2 \geqq$ $4\pi A(\rho)$ we obtain the following important inequality for the first integral occurring in (5) which we denote also by $P(\rho)$, $\rho > 0$;

$$P(\rho) = \int_{u\,=\,\rho} G\, ds \geqq \frac{4\pi A(\rho)}{|A'(\rho)|}. \tag{6}$$

Obviously

$$\int_D \int | \text{ grad } u |^2 \, d\sigma = \int_D \int G^2 \, d\sigma = \int_0^1 \int_{u = \rho} G^2 \, ds \cdot \frac{d\rho}{G} =$$

$$\int_0^1 P(\rho) d\rho. \quad (7)$$

4.   Now we define the function $\bar{u} = f(\rho)$ as follows:

$$f(\rho) = \int_0^\rho (P(t))^{1/2} \left(\frac{| A'(t) |}{4\pi A(t)} \right)^{1/2} dt, \qquad \rho > 0. \qquad (8)$$

This integral exists since $P(\rho)$ [see (7)] and $A'(\rho)$ are integrable.   Also it tends to zero with ρ so that $\bar{u}$ satisfies the boundary condition.   We have $A'(\rho) \, d\rho = 2\pi R \, dR$, hence

$$| \text{ grad } \bar{u} |^2 = \left(\frac{d\bar{u}}{dR} \right)^2 = (f'(\rho))^2 \left(\frac{d\rho}{dR} \right)^2 = (f'(\rho))^2 \frac{4\pi^2 R^2}{(A'(\rho))^2} =$$

$$(f'(\rho))^2 \frac{4\pi A(\rho)}{(A'(\rho))^2} = \frac{P(\rho)}{| A'(\rho) |}. \qquad (9)$$

The area between the circles of radii R and $R + dR$ is $2\pi R \, dR = | A'(\rho)| \, d\rho$ so that we find

$$\int_D \int | \text{ grad } \bar{u} |^2 \, d\sigma = \int_0^1 P(\rho) d\rho \qquad (10)$$

which proves indeed (1).

On the other hand we conclude from (8), by (6),

$$f(\rho) \geq \int_0^\rho dt = \rho. \qquad (11)$$

Hence

$$\int_0^1 \rho^2 | A'(\rho) | \, d\rho \leq \int_0^1 (f(\rho))^2 | A' | (\rho) \, d\rho \qquad (12)$$

which proves (2).

This establishes the assertion.

III.   Problem (b):   *Clamped Plate.*—1.   We denote the minimizing function again by u and *assume* that $0 \leq u \leq 1$ holds throughout the domain D.   Let $0 < \rho < 1$.   We consider the open set $u < \rho$ which consists in general of several simply or multiply connected components $K_\rho, K_\rho', \ldots$. One of them, say K_ρ, has the curve C ($u = 0$) as part of its boundary.   On the rest of the boundary of $u < \rho$ we have $u = \rho$.   We denote by $A(\rho)$ the area of the complementary set characterized by the condition $u \geq \rho$. The function $A(\rho)$ is continuous and monotonically decreasing; $A(0)$ is the area of the given domain D and $A(1) = 0$.

2.   We write again $| \text{ grad } u | = G$.   There is no need for any change in the argument of II 3; in particular II (4) and inequality (5) hold. Here $L(\rho)$ is the total length of the set of curves $u = \rho$.   Now we apply

the isoperimetric inequality $(L(\rho))^2 \geqq 4\pi A(\rho)$. Obviously more than this is true: $L(\rho)$ can be replaced by the total length $L_1(\rho)$ of the curves which together with C bound K_ρ. Also $A(\rho)$ can be replaced by the area $A_1(\rho)$ bounded by the curves just mentioned. We have $L(\rho) \geqq L_1(\rho)$, $A(\rho) \leqq A_1(\rho)$.

Thus II (6) holds without any change.

3. Now we introduce the notation, $\rho > 0$,

$$\int_{u - \rho} (\nabla^2 u)^2 \frac{ds}{G} = Q(\rho) \tag{1}$$

so that

$$\int \int (\nabla^2 u)^2 \, d\sigma = \int_0^1 \int_{u - \rho} (\nabla^2 u)^2 \frac{ds}{G} \, d\rho = \int_0^1 Q(\rho) d\rho. \tag{2}$$

To be sure, $Q(\rho)$ has no meaning for $\rho = 0$ but the integral (2) is finite. By Schwarz's inequality:

$$\int_{u - \rho} (\nabla^2 u)^2 \frac{ds}{G} \int_{u - \rho} \frac{ds}{G} \geqq \left(\int_{u - \rho} \nabla^2 u \frac{ds}{G} \right)^2 \tag{3}$$

or

$$(Q(\rho))^{1/2} |A'(\rho)|^{1/2} \geqq \int_{u - \rho} \nabla^2 u \frac{ds}{G}. \tag{4}$$

The integral of the function on the left-hand side of (4) exists in $0 \leqq \rho \leqq 1$ since $Q(\rho)$ and $A'(\rho)$ are integrable. Integrating we obtain

$$\int_0^\rho (Q(t))^{1/2} |A'(t)|^{1/2} \, dt \geqq \int_0^\rho \int_{u - t} \nabla^2 u \frac{ds}{G} \, dt =$$

$$\int \int_{0 \leqq u \leqq \rho} \nabla^2 u \, d\sigma = - \int_{u - 0, \, u - \rho} \frac{\partial u}{\partial n} \, ds = - \int_{u - \rho} \frac{\partial u}{\partial n} \, ds =$$

$$\int_{u - \rho} G \, ds = P(\rho) \geqq \frac{4\pi A(\rho)}{|A'(\rho)|}. \tag{5}$$

The integration indicated by the condition $u = \rho$ has to be extended over a set of curves $u = \rho$ and the normal is directed in each case into the interior of the domain $0 < u < \rho$.

4. We define now the function $f(\rho)$ in the following way. Let

$$g(\rho) = \frac{|A'(\rho)|}{4\pi A(\rho)} \int_0^\rho (Q(t))^{1/2} |A'(t)|^{1/2} \, dt, \quad \rho > 0, \tag{6_1}$$

and

$$f(\rho) = \int_0^\rho g(\rho) d\rho, \quad f(0) = 0. \tag{6_2}$$

The integral in (6_2) exists since $(0 < \rho_0 < \rho)$

$$\int_{\rho_0}^{\rho} -A'(\rho)d\rho \int_0^{\rho} (Q(t))^{1/2} (-A'(t))^{1/2} dt$$

$$= A(\rho_0) \int_0^{\rho} (Q(t))^{1/2} (-A'(t))^{1/2} dt - A(\rho) \int_0^{\rho} (Q(t))^{1/2} (-A'(t))^{1/2} dt$$
$$+ \int_{\rho_0}^{\rho} A(\rho)(Q(\rho))^{1/2} (-A'(\rho))^{1/2} d\rho.$$

In view of (5) we have $g(\rho) \geqq 1$, $f'(\rho) \geqq 1$ so that we obtain the important inequality

$$f(\rho) \geqq \rho, \qquad 0 \leqq \rho \leqq 1. \tag{7}$$

5. We determine $R = R(\rho)$ as in I, $A(0) = \pi R_0^2$ being the area of D. We consider the function $\tilde{u} = \tilde{u}(R) = f(\rho)$ defined on the circle $\tilde{D}$ of radius R_0. This function satisfies the boundary conditions

$$(\tilde{u})_{R = R_0} = f(0) = 0,$$
$$\left(\frac{\partial \tilde{u}}{\partial n}\right)_{R = R_0} = \lim_{R \to R_0} {}^u{}'(R) = \lim_{\rho \to 0} f'(\rho) \frac{2\pi R}{|A'(\rho)|} = 0, \tag{8}$$

in view of (6_1).

We have now

$$\nabla^2 \tilde{u} = \frac{1}{R} \frac{d}{dR} \left(R \frac{d\tilde{u}}{dR}\right) = \frac{4\pi}{A'(\rho)} \frac{d}{d\rho} \left(\frac{A(\rho)}{A'(\rho)} f'(\rho)\right) = \frac{(Q(\rho))^{1/2}}{|A'(\rho)|^{1/2}} \tag{9}$$

and we find

$$\int_{\tilde{D}}\int (\nabla^2 \tilde{u})^2 \overline{d\sigma} = \int_0^{R_0} (\nabla^2 \tilde{u})^2 2\pi R \, dR = \int_0^1 \frac{Q(\rho)}{|A'(\rho)|} |A'(\rho)| d\rho. \tag{10}$$

Comparing this with (2) we see that this process did not change the integral in the numerator of I (b).

On the other hand, the integral in the denominator will be for the circular domain $\tilde{D}$:

$$\int_0^1 (f(\rho))^2 |A'(\rho)| d\rho \geqq \int_0^1 \rho^2 |A'(\rho)| d\rho. \tag{11}$$

This completes the proof.

IV. Problem (c): Buckling of a Plate.—1. Dealing with problem (c), the previous argument needs only slight modifications. We define $f(\rho)$ in the same way as in III so that the integral in the numerator of I (c) does not change.

As to the integral in the denominator we have by II (7):

$$\int_D\int |\text{grad } u|^2 d\sigma = \int_0^1 P(\rho) d\rho \tag{1}$$

On the other hand [cf. II (9)]

$$| \text{ grad } \bar{u} |^2 = (f'(\rho))^2 \frac{4\pi A(\rho)}{(A'(\rho))^2},$$ (2)

and the area between the circles R and $R + dR$ is $| A'(\rho) |$ $d\rho$ so that

$$\int_D\int | \text{ grad } \bar{u} |^2 \overline{d\sigma} = \int_0^1 (f'(\rho))^2 \frac{4\pi A(\rho)}{| A'(\rho) |} d\rho.$$ (3)

But

$$f'(\rho) \geqq \frac{| A'(\rho) |}{4\pi A(\rho)} P(\rho) \geqq 1.$$ (4)

so that

$$\int_{\bar{b}}\int | \text{grad } \bar{u} |^2 \overline{d\sigma} \geqq \int_0^1 f'(\rho) \frac{4\pi A(\rho)}{| A'(\rho) |} d\rho \geqq \int_0^1 P(\rho) d\rho =$$
$$\int_D\int | \text{ grad } u |^2 d\sigma.$$ (5)

This establishes the assertion.

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[2] Lord Rayleigh, *The Theory of Sound*, 2nd ed., vol. 1, 1894, p. 345; Faber, G., "Beweis, dass unter allen homogenen Membranen von gleicher Fläche und gleicher Spannung die kreisförmige den tiefsten Grundton gibt," *Sitsber. Bayer. Akad.*, 1923, 169–172; independently, a proof was given by Krahn, E., "Über eine von Rayleigh formulierte Minimaleigenschaft des Kreises," *Math. Ann.*, 94, 97–100 (1924).

SPHERE-GEOMETRICAL UNITARY FIELD THEORY

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A conformal relativity theory has long been longed for. Now I have arrived at the following results:

1. The Kaluza-Klein space[1,2] is equivalent to the Einstein space $V_4(R^{ij} = \frac{1}{2} g^{ij} R)$ (special) dual-conformal (i.e., N.E.-Laguerre connection) geometrically so that the points in V_4 correspond to the generalized hyperspheres whose developments in the N.E. tangential spaces are hyperspheres of equal radii.

2. The Einstein-Mayer space[3] is equivalent to the Einstein space V_4 (special) Laguerre connection geometrically so that the points in V_4

correspond to the generalized hyperspheres whose developments in the Euclidean tangential spaces are hyperspheres of equal radii.   (This fact was suggested by Y. Tomonaga.[16])

3.   The Hoffmann space of the first kind,[4] which is a generalization of the Kaluza-Klein space, is equivalent to the Einstein space (special) Lie connection geometrically so that the points in the Einstein space correspond to the special linear hypercomplexes of generalized hyperspheres, whose developments in the N.E. tangential spaces are hyperspheres of equal radii.

4.   The Hoffmann space of the second kind,[4] which is a generalization of the Einstein-Mayer space, is equivalent to the Einstein space (special) parabolic Lie connection geometrically so that the points in the Einstein space correspond to the special linear hypercomplexes of generalized hyperspheres, whose developments in the tangential Euclidean spaces are hyperspheres of equal radii.   Thereby the parabolic Lie's geometry, which is quite new, is situated among others as follows:

(Dual-conformal geometry):   (Laguerre's geometry)
            $=$ (Lie's geometry): (parabolic Lie's geometry).

In the two cases 4 and 5 the said hypercomplexes are of equal generalized radii.

Our results seem to answer the question of four-dimensionality of the unitary field theory completely and to be very suggestive for its expected future developments (e.g., for the case of that including the meson field).[17,18] The conformality long longed for seems to correspond to the similarity character of the Laguerre transformations in the wider (i.e., equiform) sense.

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ERRATA

In the article "On the Interpretation of Multi-Hit Survival Curves," these PROCEEDINGS, pages 696–712, December, 1949, equation (21) on p. 702 should read

$$S = \prod_{i=1}^{m} [1 - (1 - e^{-k t D})] \tag{21}$$

and equation (22), same page, should read

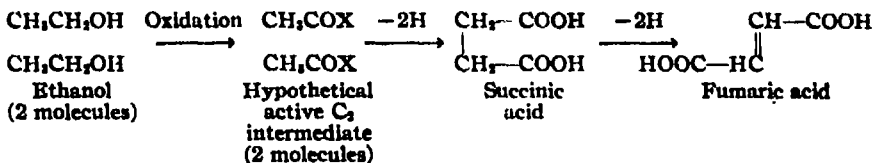$$S = [1 - (1 - e^{-kD})]^m \tag{22}$$

K. C. ATWOOD

METABOLIC EXCHANGE OF CARBON DIOXIDE WITH CARBOXYLS AND OXIDATIVE SYNTHESIS OF C_4 DICARBOXYLIC ACIDS*

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Recently[1] we advanced evidence that fumaric acid synthesis from ethanol in strain No. 45 of the mold *Rhizopus nigricans* takes place by direct condensation of two C_2 moieties according to this scheme:

$$CH_3CH_2OH \xrightarrow{\text{Oxidation}} CH_3COX \xrightarrow{-2H} \begin{array}{c} CH_2\text{---}COOH \\ | \\ CH_2\text{---}COOH \end{array} \xrightarrow{-2H} \begin{array}{c} CH\text{---}COOH \\ \| \\ HOOC\text{---}HC \end{array}$$

CH₃CH₂OH   CH₃COX

Ethanol (2 molecules)   Hypothetical active C_2 intermediate (2 molecules)   Succinic acid   Fumaric acid

The main findings were: *Specific* activity of the fumaric acid methine groups was exactly equal to that of the ethanol methyl groups; one mole of fumaric acid formed from methyl C^{14}-labeled ethanol contained *more* than the total radioactivity of the two moles of substrate ethanol; the carboxyl groups of fumarate contained all the radioactivity in excess of that in the two moles of ethanol utilized in the synthesis; some of the substrate ethanol was oxidized to CO_2 and, therefore, contained substantial radioactivity which accumulated in the closed system.

A mole of fumaric acid formed from carbinol C^{14}-labeled ethanol contained less radioactivity than the two moles of substrate ethanol; there was no radioactivity in the methine groups of the fumaric acid; the carboxyls contained all the radioactivity; radioactive CO_2 was generated by complete oxidation of a portion of the substrate alcohol, and it accumulated in the closed system.

The foregoing was interpreted to mean that the fumaric acid produced at any instant from methyl-labeled ethanol would have zero radioactivity in the carboxyl groups. However, this fumaric acid is in metabolic

equilibrium with malic and oxalacetic acids which are known to undergo reversible enzymatic decarboxylation by specific enzymes present in this organism.[2-4] Respiratory carbon dioxide liberated by decarboxylation of the initially unlabeled carboxyl groups mixes with radioactive carbon dioxide in the vessel atmosphere so that during the reversible decarboxylation, the C_4 acid now acquires radioactivity in the carboxyls.

The carboxyl groups of the fumaric acid formed at any instant from carbinol-labeled ethanol would contain radioactivity equal to the carbinol group of the ethanol. However, the carbon dioxide in the atmosphere here originates from both carbons in the ethanol, and also from endogenous respiration of the mold, hence has a specific radioactivity much less than the carboxyls of the newly formed fumarate molecules. Consequently, during reversible decarboxylation the carboxyls become diluted with carbon dioxide of lower specific radioactivity, thereby accounting for the presence of less radioactivity in the fumarate than was contained in the two alcohol molecules generating it. That nonradioactive carboxylic acids can acquire radioactivity in the carboxyl groups from labeled radioactive carbon dioxide during the action of the corresponding decarboxylases has been convincingly demonstrated in the instances of oxalacetic acid and α-ketoglutaric acid.[4,5]

A great many experiments on intermediary respiratory metabolism in which the carbon from labeled carbon dioxide was found in carboxylic acids have been interpreted[6-11] on the assumption that carbon dioxide fixation (Wood-Werkman reaction) actually provides a mode of net synthesis of the acids (over-all gain in amount of C_4 acids). Fumaric acid synthesized by *R. nigricans* from inactive ethanol in the presence of $C^{14}O_2$ contains C^{14} in the carboxyl groups,[1] a finding seemingly in harmony with the numerous above-mentioned experiments of this type which have been interpreted as a *net* synthesis utilizing carbon dioxide. However, our previous experiments with labeled ethanol make it reasonably certain that the fumarate originated by a mechanism independent of carbon dioxide fixation. In consequence of the universal character of reversibility of enzyme action, the $C^{14}O_2$-inactive ethanol experiment would seem to represent metabolic exchange between carboxyl and respiratory carbon dioxide and thus support our interpretations relative to the distinctive changes observed in the carboxyls of fumaric acid produced from *labeled* ethanol.

Although, as discussed below, a different mechanism could account for some of the observed results, the possible broader significance for tracer studies implied by the discovery of this carbon dioxide metabolic exchange with preformed C_4 acids makes additional study of the problem desirable.

Methods.—General methods and materials were precisely as described previously[1] except that the experimental vessels were 400-ml. beakers

instead of 250-ml. Erlenmeyer flasks in order to facilitate physical gas exchange.

Experiments.—Carbon dioxide being a reactant in the system, C_4 acid $\xrightarrow{\text{decarboxylase}}$ C_3 acid $+ CO_2$, its partial pressure would be expected to influence the carboxyl labeling, and these experiments center on this point. In our previous experiments the respiratory carbon dioxide was allowed to accumulate in the closed vessels (10-liter desiccators) for the duration of the experiment; this reservoir of (radioactive) carbon dioxide was assumed to be that utilized in the exchange reactions. Starting with normal air atmosphere and 25 ml. of 2% methyl-labeled ethanol, the carbon dioxide concentration in the gas phase reached 1 to 1.5% in 4 to 5 days under these conditions.

In this work a similar normal air treatment was set up as the reference point. Three other methyl-labeled ethanol treatments using portions of the same batch and amount of mycelium were run at the same time. They were identical in every respect except for differences in carbon dioxide tensions.

Treatment: (1) Ordinary air atmosphere; respiratory CO_2 allowed to accumulate. (2) Strong NaOH to absorb respiratory CO_2 keeping the CO_2 content of the gas phase nil. (3) 5% CO_2 containing 1.47 atom per cent $C^{13}O_2$; 20% O_2; 75% N_2. (4) 15% CO_2; 20% O_2; 65% N_2.

In treatment (2) the NaOH (30 ml. 6 N) was contained in a 50-ml. beaker suspended rigidly inside the 400-ml. beaker holding the culture and just above the surface of the culture liquid. The object here was to insure immediate absorption of CO_2 produced as close as possible to the surface of the liquid. Fluted filter paper in the alkali beaker increased the absorptive surface. The alkali beaker was fixed in position by fusing it to the larger beaker with thin glass rods. Treatment (3) was designed to increase the rate of exchange between carbon dioxide and carboxyl as a result of increased tension of carbon dioxide (5%). An additional quantitative check on entrance of extracellular carbon dioxide into carboxyl groups was obtained by adding $C^{13}O_2$ to the gas phase. A carbon dioxide concentration of 5% also minimizes concentration changes due to evolution of respiratory carbon dioxide, which otherwise would complicate the quantitative aspects of $C^{13}O_2$ utilization. The carbon dioxide content of the gas phase was considered to have remained constant in this experiment (less than 20% increase). Another purpose of treatment (3) was to have the $C^{14}O_2$ of respiration substantially diluted with the 5% nonradioactive carbon dioxide present in the atmosphere, resulting in a lowered specific radioactivity of the extracellular carbon dioxide available for exchange with the carboxyls of the C_4 dicarboxylic acids. Theoretically, this would reduce appreciably the specific activity of the carboxyls as compared to treatment (1).

Treatment (4) with a 15% carbon dioxide atmosphere was designed to obtain the maximum dilution of radioactive respiratory carbon dioxide, thereby achieving the lowest possible radioactivity in the carboxyls. Preliminary experiments indicated fumaric acid formation from ethanol is completely inhibited when the carbon dioxide tension exceeds 25% and about one-half normal yields are obtained in 15% carbon dioxide. Consequently, treatment (4) was set up in duplicate desiccators to obtain sufficient fumaric acid for isolation and degradation.

The desiccators containing the beakers fixed in position were shaken on a machine at 28° C. for 4 days. Analyses on the clear filtrates gave the following data:

	FINAL PH	TOTAL FUMARIC ACID FORMED, MG.
Treatment (1)	2.9	69
(2)	2.9	43
(3)	2.9	54
(4)	$\begin{cases} 3.25 \\ 3.15 \end{cases}$	$\left.\begin{matrix} 21\ 6 \\ 20.1 \end{matrix}\right\}$ 41.7

Crystalline fumaric acid was recovered from treatments (3) and (4) essentially by the procedure described previously;[1] yields were 39 and 18 mg., respectively. The fumaric acid in treatments (1) and (2) was obtained by continuous ether extraction. The fumaric acid was dissolved in water after evaporation of the ether; titrimetric estimation of the fumaric acid was made on these aqueous solutions. Other acids are not present in amounts exceeding 1% of the fumaric acid. These two solutions and the two crystalline fumaric acid preparations were then examined for specific radioactivity of the carboxyl groups as the criterion of metabolic exchange of carbon dioxide with carboxyls.

The following procedure was developed for the advantage it has of being specific for carboxyl groups of fumaric acid (and acids convertible to fumaric acid, especially succinic acid). Approximately 0.33 mM. fumaric acid in 40-ml. $M/10$ phosphate buffer at pH 7.4 was treated with 10 g. of enzyme solids of a fumarase preparation from beef heart. This preparation is identical with the succinoxidase preparation ordinarily used for manometric estimation of succinic acid.[1] During shaking at 37° C. for 1 hour, the bulk of the fumarate is converted to l-malic acid. In a test run in which residual fumaric acid was determined by hydrogenation in the presence of palladium, the final equilibrium mixture contained fumarate and malate in the ratio of 1 to 4.7. The suspension of enzyme solids was centrifuged, the cloudy supernatant acidified with H_2SO_4 to ~pH 1 and the organic acids extracted with ether for 3 days in a Kutscher-Steudel apparatus. After evaporation of the ether, the acids were taken up in

water and adjusted to pH 5.5.   A blank consisting of enzyme acting on buffer alone was carried along exactly as the test run.

The malic acid was then decarboxylated by a suspension of *Lactobacillus casei* yielding lactic acid and carbon dioxide quantitatively.[13] Starting with known amounts of *l*-malate, we have confirmed the stoichiometry of this decarboxylation, and have demonstrated its specificity particularly with respect to related acids potentially present as impurities in biochemical work of this kind.   Under the same conditions that one mole of carbon dioxide is liberated from malate, the bacteria were completely inert against fumarate, citrate, succinate and α-ketoglutarate, singly and in combination.

Cells from 200 ml. of malate medium (glucose 1%, *l*-malate 0.25%, yeast extract 0.1%, phosphate buffer pH 6.6, 0.5%) were centrifuged after 40 hours' incubation at 28° C., washed twice in $M/10$ phosphate buffer at pH 5.5 and suspended in 3 ml. of the same phosphate buffer.   This thick suspension was placed in one side arm of a large (160-ml.) Warburg vessel and 1.0 ml. of 10 N H_2SO_4 in the second side arm.   The solution containing malic acid at pH 5.5 ($\sim$40 ml.) was in the main chamber. After temperature equilibration, the bacteria and the malate solution were mixed; carbon dioxide evolution is virtually complete in about 1 hour, after which bound CO_2 is liberated by the H_2SO_4.   Later it was found that the decarboxylation is fully effective at pH 4, obviating the need for the acid dump.   In the test run cited above, where malate was generated from fumarate by fumarase, recovery of C_4 acids was 100%.   Fumaric acid taken $= 0.40$ mM.; fumaric acid left, determined by hydrogenation $= 0.07$ mM.; malic acid, by difference $= 0.33$ mM.; malic acid determined by bacterial decarboxylation $= 0.33$ mM.

The ether extraction is an essential step in this determination.   The cloudy supernatant from the fumarase treatment retains considerable amounts of apoenzymes of oxidation systems and these are activated by coenzymes leaching from the bacterial cells; complete oxidation of the residual fumarate to carbon dioxide follows, yielding fallacious results. Curiously, malate itself is not oxidized under these conditions notwithstanding the fact that fumarate oxidation might be expected to proceed via malate.   Carbon dioxide from a bacterial blank on buffer alone generally is a negligible proportion of the test runs.

The carbon dioxide from malate decarboxylation is collected in an evacuated 500-ml. bulb containing CO_2-free alkali.   The bulb is attached to one venting plug of the Warburg vessel, which is flushed by carbon dioxide-free air down through the vessel from the other venting plug. The carbon dioxide is then precipitated as $BaCO_3$, digested on a waterbath, filtered onto tared porcelain counting disks, dried, weighed and taken for radioactivity measurements.   This procedure recovers, as $BaCO_3$,

in excess of 95% of the manometrically determined CO_2. All radioactivity results are expressed as specific activity (counts/sec./mg. $BaCO_3$). After radioactivity measurements, the C^{13} in the $BaCO_3$ from treatment (3) was analyzed in a mass spectrometer.

Results.—Because of the limited amounts of fumaric acid obtained in each of the four treatments, specific activity measurements on an aliquot of each fumarate were not attempted. Also, they are unnecessary for this work in which the specific activity of only the carboxyl groups is of importance. However, we have repeatedly shown that mixing of methine and carboxyl groups of fumaric acid (produced from methyl and carbinol of ethanol) does not occur (see below) and that the specific activity of the methine group is precisely that of the methyl group of the starting ethanol, and two times that of the ethanol itself. This nonmixing is all-important, for, as shown below, it means that carbinol-C cannot become methine-C

TABLE 1

SPECIFIC RADIOACTIVITIES OF CARBOXYL GROUPS OF FUMARATE PRODUCED BY *Rhizopus nigricans* UNDER DIFFERENT CO_2 TENSIONS[a]

TREATMENT		SPECIFIC RADIOACTIVITY OF CARBOXYL GROUPS[b]
(1)	Air atmosphere[c]	1.06
(2)	No CO_2(NaOH)[d]	1.11
(3)	5% CO_2 containing $C^{13}O_2$[e]	0.91
(4)	15% CO_2	0.80

[a] Cultures consisted of mycelium suspended in 25 ml. 2% methyl C^{14}-labeled ethanol.

[b] Counts/sec./mg. $BaCO_3$. Corrected for carbon dioxide from blank carried from fumarase stage through entire analytical process.

[c] Carbon dioxide allowed to accumulate in desiccator. Concentration of CO_2 in gas phase at end of incubation = 1.5%.

[d] By analysis there was no CO_2 in the atmosphere.

[e] See data on $C^{13}O_2$ below

of fumarate, but that methyl-C (of ethanol) can become carboxyl-C of fumarate. Table 1 gives the carboxyl data.

Discussion.—The fact that elevated carbon dioxide tensions caused definite dilution of the radioactive carboxyl groups, and in proportion to the carbon dioxide tension, proves that carbon dioxide itself influences in some way the radioactivity of the carboxyls. A not implausible interpretation is that carbon dioxide is metabolically exchangeable with carboxyls, presumably through the action of decarboxylases. Absolute proof is perhaps provided by the fact that extracellular $C^{13}O_2$ is actually found in fumarate carboxyls. Fumarate itself having been established[1] to arise by a $2C_2$ condensation would appear to rule out the entrance of $C^{13}O_2$ via the Wood-Werkman reaction in the conventional sense of *net* synthesis of the C_4 carbon chain from $C_3 + C_1$ moieties.
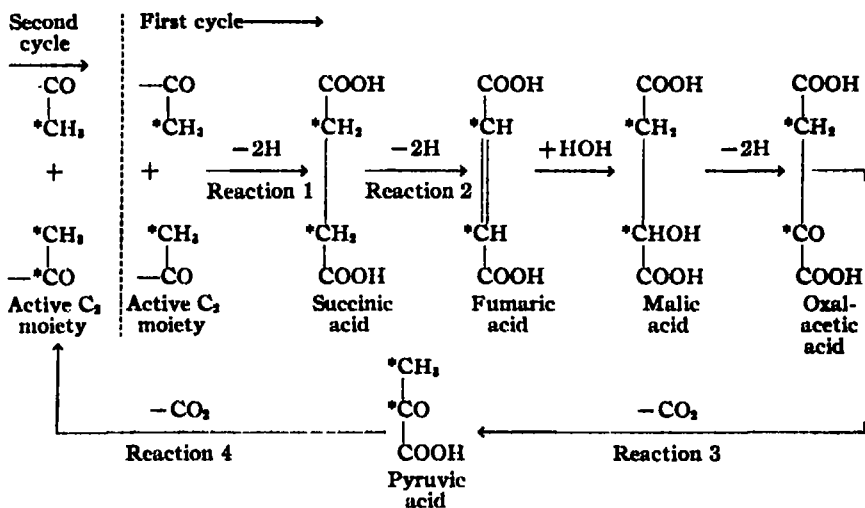
Calculation of Extracellular Carbon Dioxide Exchange with Carboxyls.—

The C^{14} content of the isolated fumaric acid in treatment (3) was found to be 1.13 $\pm$ 0.005 atom per cent.[14] 1.13 $-$ 1.09 (C^{14} content of normal CO_2) $=$ 0.04% excess C^{14} in the CO_2 obtained from total combustion of fumaric acid. Since the C^{14} is located in carboxyls only, they contained 2 $\times$ 0.04 $=$ 0.08% excess C^{14}.

The C^{14} content of the initial gas phase was found to be 1.47 $\pm$ 0.007 atom per cent. 1.47 $-$ 1.09 $=$ 0.38% excess C^{14}. $\dfrac{0.08 \times 100}{0.38} = 21\%$ of all the fumaric acid carboxyl groups exchanged with extracellular carbon dioxide.

Changes in Carboxyl Radioactivities in the Absence of Extra-cellular CO_2.—Respiratory Cycles: A most significant fact of this experiment is the virtual equivalence of specific activities of the carboxyl groups in the CO_2-free atmosphere NaOH (treatment 2) vs. air with accumulated CO_2 (treatment 1). This indicates that extracellular carbon dioxide exchange is not the only mechanism of incorporating radioactivity into carboxyl groups, i.e., converting methyl-C of ethanol into carboxyl-C of fumarate. Theoretically two mechanisms could operate to alter the status of carboxyls in the absence of extra-cellular carbon dioxide, and a strong possibility exists that both act simultaneously. They are: (1) exchange with intracellular respiratory carbon dioxide, of which a finite concentration must always be present as long as the fungus cells are metabolically active. In view of the demonstrated participation of extracellular carbon dioxide, it may be reasonably assumed that some internal carbon dioxide also exchanges with carboxyls before it leaves the cell; the quantitative significance of this point is a matter for the future; (2) oxidative conversions of ethanol methyl-C to fumarate carboxyl-C. Rather convincing evidence for a mechanism of this type operating over and above carbon dioxide exchange is provided by the specific activities of the carboxyls in treatments (1) and (2), and the specific activity of the respiratory carbon dioxide in treatment (1), which was determined as 0.85. Thus, if all the carboxyls in treatment (1) had exchanged with carbon dioxide to the extent that they acquired the specific activity of the gas they would still fall short of the observed carboxyl value of 1.06. Thus, a second mechanism independent of carbon dioxide-carboxyl exchange is indicated. The fact that ethanol methyl-C is converted to fumarate carboxyl-C connotes an oxidation, and the assumption that a tricarboxylic acid respiratory cycle takes place in these cells would fit the observed facts. This cycle would, however, differ from the conventional cycle in the respect that the C_4 which condenses with C_2 to generate C_6 arises by $2C_2$ condensation instead of by the generally supposed Wood-Werkman reaction. This may well prove to be one mechanism characteristic of the tricarboxylic acid cycle in carbohydrate metabolism.

Postulation of a dicarboxylic acid respiratory cycle would also fit the observed facts, and this is a distinct and attractive possibility in this instance.

Second cycle	First cycle $\longrightarrow$				
$\longrightarrow$					
$\cdot CO$	$—CO$	$COOH$	$COOH$	$COOH$	$COOH$
$^*CH_2$	$^*CH_2$	$^*CH_2$	$^*CH$	$^*CH_2$	$^*CH_2$
$+$	$+$	$-2H$ Reaction 1	$-2H$ Reaction 2	$+HOH$	$-2H$
$^*CH_2$	$^*CH_2$	$^*CH_2$	$^*CH$	$^*CHOH$	$^*CO$
$—^*CO$	$—CO$	$COOH$	$COOH$	$COOH$	$COOH$
Active C_2 moiety	Active C_2 moiety	Succinic acid	Fumaric acid	Malic acid	Oxal-acetic acid

$-CO_2$ Reaction 4 $^*CH_3$ $^*CO$ $COOH$ Pyruvic acid $-CO_2$ Reaction 3

This scheme was formulated for carbohydrate and pyruvate oxidation some 30 years ago, though without proof.[1b, 14] During the intervening years good evidence has been gathered for each of the above steps, and Krebs in 1943[10] states that "...the chief weakness of this theory was the complete lack of evidence supporting the assumption of a formation of succinate from acetate." Our data provide, therefore, the means of closing one (if not the last) gap relative to a "dicarboxylic acid respiratory cycle." Four principal steps in this cycle, namely, reactions 1, 2, 3 and 4, have already been demonstrated separately in *R. nigricans* No. 45, and if they operate in sequence, as is likely, a C_4 dicarboxylic acid cycle will in fact have been established. Important to note is the experimental fact that the fumaric acid which accumulates from ethanol in these cultures is rapidly oxidized to completion (i.e., to carbon dioxide) as soon as all the ethanol is consumed.

It can be seen from the above scheme that after the first cycle, the original methyl-labeled C_2 becomes labeled in both carbons. This C_2 labeled in both carbons mixes with more substrate methyl-labeled C_2. The C_4 acids now resulting from a $2C_2$ condensation reaction would be labeled in a manner that matches the experimentally observed labeling in two critical respects, namely, (a) the inside carbons (methine-C of fumaric acid) always show the same specific activity as the methyl groups of the starting C_2 moiety regardless of the number of cycles made; and (b) the specific activity of the carboxyl groups can never attain the specific activity

of the methine groups so long as substrate ethanol is present. During the relatively long (4 days) duration of the incubation period in this experiment it is probable that the accumulated fumaric acid at any one time represents the differential between the rates of formation and of oxidation of fumarate, both occurring concomitantly.

It is to be emphasized that a tricarboxylic acid cycle would yield results exactly as the dicarboxylic acid cycle and at present it is impossible to discriminate between these two possibilities or to exclude the possibility that both function simultaneously.

Confirmation of the Synthesis of Fumaric Acid by 2C₂ Condensation.· – Because the validity of many of our conclusions depends on the absence of any significant mixing of methine-C (methyl-C of ethanol) and carboxyl-C (carbinol-C of ethanol), we have re-examined this matter more carefully since our first published experiments. Three independent methods of degradation have now been used. One was, in essence, the formic acid procedure described in our previous paper; this determines specific activity of methine carbons of fumaric acid. A specimen of fumaric acid originating from carbinol-labeled ethanol, and having a specific activity of 0.30 was oxidized with acid-permanganate and the formic acid (over-all yield = 89%) in the fourth quarter of a Duclaux distillation taken for radioactivity measurements. The carbon dioxide from oxidation of this formic acid was trapped as $BaCO_3$, which was weighed, and the carbon dioxide liberated for gas ionization measurements with a vibrating reed electrometer. The results were expressed as amperes of ion current per milligram of original $BaCO_3$. Similar measurements were made on the total carbon dioxide from complete oxidation of a portion of the fumaric acid itself. Total fumaric acid = 14.5×10^{-16} amperes net per milligram $BaCO_3$. Formic acid = 0.037×10^{-16} amperes net per milligram $BaCO_3$. The actual amperage here was 1.7 times background. The following expression gives the value for the carboxyl-carbons of the fumaric acid: $14.5 \times 10^{-16} = \dfrac{2(0.037 \times 10^{-16}) + 2X}{4}$; $X = 28.96 \times 10^{-16}$ amperes per milligram $BaCO_3$ from carboxyl-carbon. Ratio of carboxyl-carbon to methine-carbon $= \dfrac{28.96 \times 10^{-16}}{0.037 \times 10^{-16}} = \dfrac{783}{1}$. Thus, the mixing of carboxyl-carbon at most could be 1 part in 783, an insignificant amount. In fact, the steam distillate contained some very small amount of volatile radioactivity which did not follow the formic acid Duclaux curve, and in all probability even the minute activity recorded as formate was due to an impurity and not to formic acid itself.
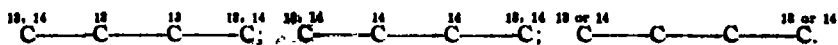
The second method of degradation consists of measuring the CO_2 from decarboxylation of malate obtained from the above fumaric acid of specific

activity 0.30. The specific activity of this CO_2 was 0.62. Theory = 0.60 (calculated from specific activity of total fumaric acid, which was 0.80, as noted above the methine-C in this fumaric acid contained no radio-activity).

The third method of degradation (details of which will be given in a later publication) employs these principles: fumaric acid $\xrightarrow{\text{fumarase}}$ malic acid $\xrightarrow[L.\ casei]{-CO_2}$ lactic acid $\xrightarrow[H_2SO_4]{KMnO_4}$ acetic acid $+$ CO_2. The acetic acid-carbons represent both methine-carbons of fumarate. The specific activity of methine-carbons of standard fumaric acid sample as determined by the above acetate method was 4.8; that by the formate method on the same sample was 4.8. Thus, three independent methods of degradation agree on the specific activity composition of fumaric acid.

Oxidative Synthesis of C_4 Dicarboxylic Acids and Labeled CO_2 Fixation Experiments.—If our interpretation of the data obtained relative to $2C_2$ condensation is correct in its main conclusion, it means a direct oxidative pathway to the C_4 dicarboxylic acids exists in addition to the Wood-Werkman reaction. Indeed, demonstration in normal tissues of the Wood-Werkman formation of C_4 acids by locating the labeled CO_2 in carboxyls of these acids would not, in view of our experiments, constitute unequivocal proof of *net* synthesis involving carbon dioxide, for metabolic exchange of the carbon dioxide with carboxyls of acids *preformed, and by a route independent of CO_2 fixation* may yield the same results. It would seem that in the absence of evidence excluding the operation of other mechanisms of organic acid synthesis, the incorporation of labeled carbon dioxide into carboxyl groups would have to be interpreted with considerable reserve.

Biosynthesis of Doubly Labeled C_4 Dicarboxylic Acid Tracers.—The results obtained in treatment (3) in the experimental section suggest a useful method of securing labeled C_4 dicarboxylic acids, whose isotopic composition allows the fate of carboxyl-carbons and central-carbons to be followed independently. By using the appropriate methyl-labeled ethanol and the appropriate labeled CO_2, three different kinds of C_4 acids can be produced:

$$\overset{12,\ 14}{C}\!-\!\overset{12}{C}\!-\!\overset{12}{C}\!-\!\overset{12,\ 14}{C};\quad \overset{12,\ 14}{C}\!-\!\overset{14}{C}\!-\!\overset{14}{C}\!-\!\overset{12,\ 14}{C};\quad \overset{12}{C}\!-\!\overset{14}{C}\!-\!\overset{14}{C}\!-\!\overset{12\ or\ 14}{C}.$$

Summary.—Biosynthesis of fumaric acid by a $2C_2$ condensation in the mold *R. nigricans* has been confirmed and extended. It has been demonstrated that CO_2 can undergo metabolic exchange with carboxyls of pre-formed fumaric acid. According to these experiments demonstration in normal tissues of the Wood-Werkman formation of C_4 dicarboxylic acids by locating the labeled CO_2 in carboxyls of these acids would not con-stitute unequivocal proof of *net* synthesis involving carbon dioxide. Evi-

dence has been obtained for the probable operation of a C_4 dicarboxylic acid respiratory cycle in this mold.

† On leave of absence from the University of Texas.

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OBSERVATIONS ON THE EFFECTS OF MALEIC HYDRAZIDE ON FLOWERING OF TOBACCO, MAIZE AND COCKLEBUR*

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In April, 1949, experiments were begun in this laboratory to determine effects of maleic hydrazide[1] on growth of a number of species of plants. Our first objective was to determine its effect upon growth and differentiation of Turkish tobacco plants which were just producing flower buds. Concentrations ranging from 0.05 to 0.8 per cent were used in treating five lots of ten plants each. The solutions were made up in distilled water to which one drop of Aerosol OT was added per 100 ml. to act as a spreading agent. All leaves of the plants were then sprayed on both upper and lower surfaces. Each plant received approximately 25 ml. of the proper solution. Height measurements were made and note taken of the number of flower buds visible without the aid of a hand lens. The plants were then placed on the greenhouse bench in such a manner that light and moisture conditions would be as uniform as possible.

TABLE 1

Effect of Five Concentrations of Maleic Hydrazide on Growth in Height and Flower Expression in Turkish Tobacco

PER CENT MALEIC HYDRAZIDE USED	NO. OF PLANTS TREATED	AVERAGE HEIGHT, CM., APRIL 19	NO. OF PLANTS WITH FLOWER BUDS, APRIL 19	AVERAGE HEIGHT, CM., MAY 17	NO. OF PLANTS WITH LIVING FLOWERS, MAY 17	AVERAGE INCREMENT IN CENTIMETERS
0.8	10	34.6	2	48.2	None	13.6
0.4	10	36.4	3	49.5	None	13.1
0.2	10	38.1	5	57.4	None	19.3
0.1	10	39.4	6	65.0	5	25.6
0.05	10	39.7	4	91.6	10	51.9
Control	10	43.0	1	106.9	10	63.9

None of the plants treated with 0.2, 0.4 and 0.8 per cent maleic hydrazide had produced new flowers and all those initiated at the time of treatment were dead. Some of these abscissed. On the other hand, of the ten plants treated with 0.1 maleic hydrazide, only five produced flowers and these grew slowly. All of those plants receiving 0.05 per cent maleic hydrazide flowered, but again the rate of development of the inflorescences was slower than the controls. Figures obtained on height increment following treatment indicate nearly equal effectiveness of concentrations of 0.2, 0.4 and 0.8 per cent maleic hydrazide in suppressing terminal growth. Lower concentrations showed decreasing effectiveness in inhibiting over-all growth in height (table 1).

Maize is also very markedly inhibited in growth by maleic hydrazide. The effective concentration range for it is approximately the same as for tobacco. Wisconsin hybrid No. 525 maize was used in the tests reported here. Single plants were grown in soil in two-gallon crocks. Seeds were planted in July and the plants were approximately one meter tall with no visible staminate inflorescence (tassel) at the time of treatment. Crystalline maleic hydrazide was used in making the solutions, and Emulphor EL was employed as the spreading agent. All solutions were adjusted to
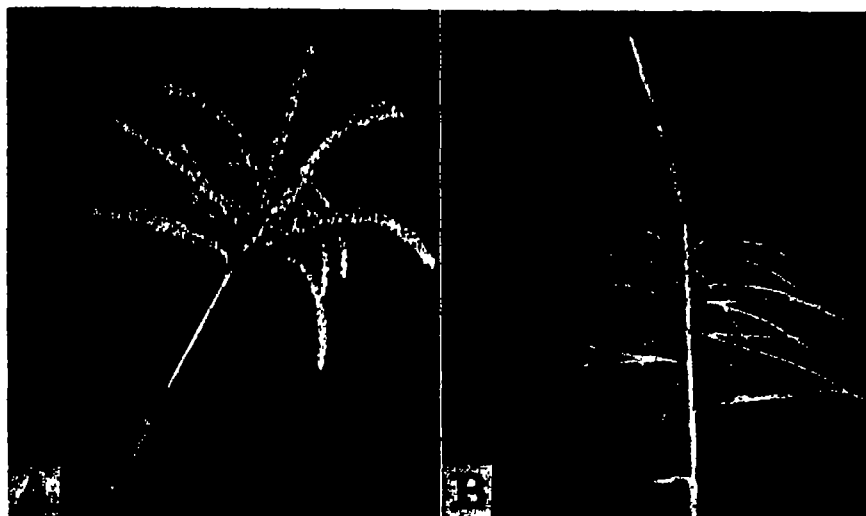

FIGURE 1

Appearance of the staminate inflorescence of maze 45 days after treatment with maleic hydrazide. *A.* Control. *B.* Leaves sprayed with 0.025% maleic hydrazide solution when the plant was 35 days old. Note the lack of anthers in the flowers of the treated plant.

pH 6.0. Concentrations ranging from 0.025 to 0.2 per cent were used. Each one exerted a marked effect.

The highest concentration prevented flower expression completely. The corn plants receiving the lowest concentration grew almost as rapidly as the controls but produced sterile tassels. The number of staminate flowers was approximately the same as in the controls but no anthers were formed (Fig. 1, B). The pistillate inflorescences on these plants, however, were composed of fertile flowers. Seed was set at the base of the ears and more would have probably been set toward the apex had the amount of fertile pollen in the greenhouse been higher.

Because of the striking effects of maleic hydrazide on flower development in tobacco and corn, it seemed desirable to determine if this substance would prevent flowering in a photoperiodically sensitive plant. *Xanthium saccharatum* Wallr.[1] was selected for this purpose because of the large body of information available about light and dark requirements for flowering. Normally Xanthium changes from the vegetative to reproductive condition when provided with cycles of 8.5 hours of dark— 15.5 hours of light.[3] Spraying the leaves with as little as 10 ml. of a 0.025 per cent maleic hydrazide solution at the time photoinduction was begun shifted the critical daylength by thirty minutes. Controls which did not receive maleic hydrazide shifted very rapidly from the vegetative to reproductive condition. On the other hand, those receiving this substance changed slowly from the vegetative condition and this held true even when the dark period given was $10^1/_2$ hours long.

Maleic hydrazide thus appears to be a good inhibitor of flower buds as well as vegetative buds.[5,6] It also causes loss of apical dominance. While it suppresses lateral buds as well as terminals much higher concentrations are required for this than for inhibition of the terminal bud alone. Although some of the leaves produced subsequent to treatment with high concentrations of maleic hydrazide show malformation, concentrations effective in inhibiting flower bud development which do not affect leaf development can be employed. This substance may possibly find application where it is necessary to delay or completely inhibit flowering as in tobacco, and possibly celery and lettuce production. Since there appears to be a difference in sensitivity of terminal and axillary flowers to this substance new possibilities in the production of hybrid seed are opened up. It is often desirable, for example, to have, in hybrid seed corn production, a pollen sterile parent, and preliminary experiments indicate this condition can be induced with the aid of maleic hydrazide.

[1] Supplied through the courtesy of Naugatuck Chemical Company, Naugatuck, Connecticut, as a 30 per cent solution in diethanol amine.

[2] This is the species Hamner and Bonner refer to as *Xanthium pennsylvanicum*. According to Parker, *et al.*,[4] this species has been identified by Dr. S. F. Blake as *X. saccharatum*.

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[4] Parker, M. W., Hendricks S. B., Borthwick, H. A., and Scully, N. J., *Ibid.*, 108, 1–26 (1946) (see p. 3).

[5] Schoene, D. L., and Hoffmann, O. L., *Science*, 109, 588–590 (1949).

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REPEATED MUTATIONS IN ONE AREA OF A MOUSE CHROMOSOME

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Several years ago it was found that the combination of two different lethal mutations in the house mouse gave rise to a balanced lethal line in which both mutations could be maintained indefinitely in heterozygous condition without selection.[1,2] This behavior was shown to be due to absence of recombination in this region of the chromosome probably due to allelism of the two mutations concerned. The system can be diagrammed as follows:
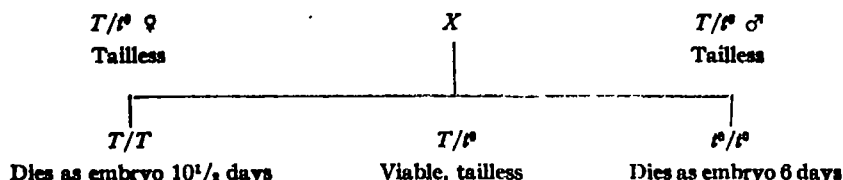

FIGURE 1

Balanced lethal line A.

T symbolizes a dominant mutation, $T/+$ having a short tail (Brachyury) while t^0 is recessive to normal and interacts specifically with T to produce the tailless phenotype. Recombination has not been observed either in matings of T/t^0 or of KiT/t^0 or FuT/t^0, Ki and Fu being dominant mutations about 4 crossover units distant from T. The mutation t^0 is thus probably connected with an inversion in this region.[10]

A similar balanced lethal tailless line, T/t^1, known as Line 29 has likewise been maintained for about 15 years.[3,4] One of the lethals is different from that in Line A, since crosses of T/t^0 by T/t^1 produce, in addition to tailless progeny T/t^0 and T/t^1, a regular proportion of *normal-tailed* offspring which when tested prove to be t^0/t^1. The mutation t^1 likewise was found to eliminate recombination over the area including the loci of T and Ki. A third line[5] (Line 19) behaved in all respects like Line 29 and contained a lethal, t^2, not distinguishable from t^1.

In inbreeding the balanced lethal lines, T/t^n, rather rare exceptional offspring were occasionally found, about a dozen during fifteen years among several thousand regular offspring. These were saved and tested

as possible evidences of recombination between T and $t^n(t^0, t^1, t^2)$. Recombination in this region should occasionally result in gametes Tt^n and $++$, which in combination with regular gametes T and t^n should give rise to two viable exceptional phenotypes: $T/++$ (Brachyury or short tail) and $t^n/++$ (normal tail).

One exception with a short tail resembling Brachyury proved to be T/t^n combined with other modifying genes for increased length of tail. The latter were later concentrated by selection in a line which bred true to the short-tail condition but which nevertheless proved to be T/t^n (either t^0 or t^1) as in the parents. It contained no new mutation at the T locus.

The normal-tailed exceptions which were tested proved not to belong to either of the recombinant types. All of those which were adequately tested proved to be t^1/t^n, that is, each contained the t-type mutation of the line in which it arose and another different mutation of the same type.

The detailed breeding data on the first of these additional mutations to be extensively analyzed are in course of publication elsewhere. Here it need only be mentioned that whether or not a normal-tailed exception from a balanced line contains a new mutation of this type can be determined by testing the exception by Brachy, $T/+$. If the exception is, for example, t^1/t^n, then two tailless lines can be derived from the tailless progeny of the test cross, i.e., T/t^1 which will balance both in inter se matings and when tested by the parent type T/t^1; and T/t^n, a new tailless line which will always give normal-tailed progeny t^1/t^n when crossed with the parental line. Whether the new mutation gives rise to a new balanced lethal line will depend on whether it is (1) lethal when homozygous and (2) fails to undergo recombination with locus T. One of the newly recognized mutations, t^3, is viable when homozygous, so that matings of tailless T/t^3 by tailless T/t^3 regularly yield tailless T/t^3 and normal (or nearly normal-tailed) progeny which can be shown to be t^3/t^3. Another exception has given rise to a new balanced lethal line T/t^4, in which t^4/t^4 dies before birth. Neither t^3 nor t^4 have shown recombination with T.

In addition to these, we have recorded other exceptions of similar origin, i.e., found as single normal-tailed individuals in the balanced lethal tailless lines. Exceptions t^2, t^3, t^5 were found in Line A (T/t^0) but the chromosomes containing them were lost before it could be determined whether they represented new t alleles or recurrences of old ones such as t^1. Exceptions t^3, t^4, t^5, t^7, t^8, t^9 and t^{10} were found in the balanced Line 29 (T/t^1). Exception t^3 was found in a nearly sterile male (t^1/t^3) and the chromosome was lost in F_3 following outcross. Exceptions 3, 4, 7, 8 and 9 were found in normal-tailed females which segregated for t^1 and another allele without recombination (t^1/t^n). Lines with the new alleles are being extracted. Exception t^{10} was found in a normal-tailed male from T/t^1 parents and is being tested.

In addition to these occurrences in our laboratory, Dr. T. C. Carter, Institute of Animal Genetics, Edinburgh, Scotland, has found tailless mice in a normal stock of mixed ancestry having no known relationship to T or t^n stocks. These proved to contain a new mutation[6] indistinguishable from T, and a t-type mutation (t^6) which is lethal. These have given rise to a new balanced lethal tailless line, Line 6 (T/t^6). Using animals kindly sent to us by Dr. Carter, we have found t^6 to be different from t^1 since t^1/t^6 is viable and normal. No combinations t^0/t^6 have yet been found (from crosses of $T/t^0 \times T/t^6$) and so t^6 is not distinguishable from t^0 and may be a recurrence of t^0.

It is apparent that inbreeding of balanced lethal lines provides an excellent method for detecting changes of the above sort and that the region involved is in fact quite mutable. We estimate that a minimum of 14 changes of the t type have occurred together with two of the T type. Earlier indications of what may have been similar changes[7, 8] are not included. The mode of origin of these changes is interesting, since in our laboratory all have been found in the offspring of parents who themselves contained a t-type chromosome (3 from T/t^0, 7 from T/t^1). We have tested many sporadic tailless mice found in our Brachy $T/+$ stocks and in outcrosses, but have never found a t-type mutation in these. The first t^0 mutant was found by Dobrovolskaia-Zavadskaia[9] after outcross of $T/+$ by a wild mouse; and t^1 and t^2 were found after outcrosses of $T/+$ to normal laboratory stocks, but since the $T/+$ stocks of Zavadskaia occasionally produced tailless animals, the prior existence of t-type chromosomes in her stocks cannot be excluded.

It is probable that t^0 and t^1 are connected with inverted sections of chromosomes IX since they prevent recombination in the region from T to Ki—about 4–6 units.[10] The question thus arises whether all those derived from t^1, (t^2, t^4, t^5, t^7, t^8, t^9, t^{10}) contain the same inversion; or whether new inversions have been induced by t^1. This point is being investigated by cytogenetic methods. Unfortunately such a study cannot be made of exceptions derived from t^0 since the few which were detected were lost.

Although the results of such a study need not be anticipated, it is simpler for the present to assume that inversion is a less frequent process than the occurrence of new alleles within an inversion, particularly since loci may become unstable in or near an inversion; and to treat the series $T \ldots t^n$ as allelic alterations of the same locus. All t mutations, it must be remembered, interact specifically with T to produce taillessness, but show no interaction[10] with the nearby loci Ki and Fu. They have certain other peculiarities in common also such as the production of male sterility[11] in compounds t^a/t^x which indicate similar interaction effects upon spermatogenesis. Extensive fertility tests on males t^0/t^2 and t^1/t^2, which will

be reported separately from this laboratory, and tests on other alleles now in progress, show that while t^n/t^F males are most often sterile, some do produce functional sperm. Such semisterile males show the same absence of recombination as occurs in t^n/t^F females. Whether the sterility is influenced chiefly by the particular alleles or by the inversions concerned remains to be determined. The male sterility is important not only as evidence of interaction among alleles but as a limitation to the recovery of new alleles which in general can only be recovered from female exceptions.

Although in these respects alterations of the T "locus" conform to those expected from alleles, there is one way in which they are quite exceptional. So far as tested the compounds containing two *different* alleles are viable even when the component alleles are lethal when homozygous. This is true of t^0/t^1, t^0/t^2, t^0/t^4, and t^1/t^4, i.e., all cases so far thoroughly tested in which both alleles are known to be lethal. As pointed out in the first description of this phenomenon of complementary action of alleles,[1] this means that there is a physiological divergence of function such that one lethal allele makes good the defect brought about by the other. This type of divergence is difficult to reconcile with any simple hypothesis of quantitative alterations of the same single element. Either several elements, each susceptible of separate alteration and of influencing different processes are held together at such a locus in an organization that is not disrupted by crossing over,[12] or else the single element is capable of a very large variety of changes leading to physiologically antithetic or compensatory changes.

Several cases of such complex loci have recently been shown to consist actually of two or more loci separable by rare crossing-over[13, 14, 15] and forming "pseudoallelic series." Other allelic series are known within which apparently qualitative changes have occurred, requiring the assumption that the gene or locus consists of more than one element.[16] This situation apparently obtains at the loci governing self-incompatibility in plants[17] and possibly at the Rh locus in man.[18] Finally, there are modifications of what appears to be a single locus which show related effects and dominance in relation to one character and compensatory or antithetic effects upon another.[19, 20] Hosino has in fact provided an exact parallel to the relations of t alleles since in the ladybird beetle he has found over 20 alleles governing elytral pattern, among them two which are lethal when homozygous but viable in compound.[20] Lewis, similarly, has shown that a self-incompatibility gene in *Oenothera organensis* may exhibit two antithetic types of specificity, and assumes that this locus consists of at least two parts.[17] These cases indicate that there may be a more or less continuous gradation of loci from those subject only to the quantitative type of change usually associated with true allelism to those in which

there are several separable elements, which have remained together after adjacent duplication or repeat formation. The T locus in the mouse probably belongs in about the middle of this spectrum together with the S locus in plants and the spotting locus in the ladybird beetle; that is, the "alleles" show rather wide physiological divergence and complementary interactions in certain of their effects, yet share similarities indicative of allelism and show no recombination.

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SPECIFICITY OF INTERACTION BETWEEN IDENTICAL MOLECULES

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Among specific interactions between molecules, there is one type which is of particular importance, and that is the specific attraction between a pair of macromolecules on condition that they are identical or almost identical. Such a phenomenon is encountered in synapsis during meiosis, conspicuously in the phenomenon of inverted synapsis, which shows that pairs of almost identical genes attract each other strongly.

The most striking example of specific attraction between identical large molecules occurs in the self-duplication process of genes, i.e., of chromosomes in mitosis. The current view about self-duplication is as follows. Starting with an original chromosome thread, one assumes that replicas of the genes contained in that chromosome are gradually being built up from the material of the cell medium. By selective attraction, the constituent molecules of the original gene collect, from the surrounding medium, molecules which happen to be identical with the former ones (and which because of their smallness compared with a gene, are readily available in the cell), to the neighborhood of the corresponding molecules of the original gene. Thereafter, chemical bonds link these molecules together, forming a daughter gene. What is so astonishing in this phenomenon is its accuracy of differentiation, which permits identical replicas to be handed down through enormous numbers of chromosome generations; its versatility, which implies that all kinds of genes and their mutants self-duplicate accurately; and the long range of the specific forces involved, just as in synapsis. The accuracy of the phenomenon is evident; any mistake in self-duplication in the sex cells would show up as a mutation, in addition to those mutations[1] which are caused by a monomolecular change of the genetic material due to radiation or temperature effects. The versatility is evidenced by the inheritability of mutations: The fact that mutated genes are accurately reproduced shows in a striking fashion how much the self-duplication phenomenon is independent of the particular molecules involved. The long range of the specific interaction forces is indicated by the fact that the pair of specifically interacting molecules will have to interact at distances at least equal to the considerable thickness of the constituent molecules and they are usually not flat. Therefore, we have to look at these phenomena as something very different from ordinary chemical bond formation.

Various theories have been advanced to explain self-duplication.[3] The duplicates will have to be assumed to be actual replicas, rather than but functionally similar molecules; otherwise, it will be an insurmountable difficulty to account for the accuracy and versatility of the phenomenon. In this respect, self-duplication of genes is quite different from other building processes in biology.

Many attempts have been made to explain specific interaction on the basis of steric effects. Again, the accuracy of specific attraction, the versatility and range of the phenomenon would be very difficult to understand on such a hypothesis. Also we would have to expect the formation of complementary structures, rather than replicas of genes: A laevo structure would be expected to build up a dextro structure.

It has been shown[3] that some special compounds can be understood to reproduce by the assumption of a chemical chain reaction process. The serious difficulty of such a theory is the impossibility to account for the versatility with which all types of genes perform the same accurate reproduction.

H. J. Muller[4] suggested the investigation of interaction of thermally excited molecule vibrations. Molecules in whose specific interaction we are interested have a well-defined structure: X-ray investigations of some proteins which are supposedly similar to constituent molecules of genes have shown such large numbers of Fourier components that even without knowing their structure we know that every atom is located at a well-defined place.[5] Also, the following argument may support this view of a rigid structure. Organic molecules in the cell usually form and break' bonds at a considerable rate. It is only molecules with well-defined structures (without possibilities of many internal rotations) which have a high probability that any broken bond will properly heal up again, without some foreign molecules getting a chance to join in at a break. Thus, a necessary condition for the outstanding stability of the genes is that the material of which the genes are built have a rigidly defined structure, say, as in a three-dimensional framework. The amplitudes of the vibrations are of the order of 10^{-9} cm., a figure which depends but little upon whether we consider the lowest or highest vibrations in the molecule. The highest wave numbers are well above $kT/hc \sim 200$ cm.$^{-1}$, that is in the domain where quantum effects are important. We know that molecular vibrations are usually but little perturbed by the environment in which the molecule is placed. Let us study coupled thermal vibrations of a pair of identical molecules and investigate them as a possible cause of their specific attraction. As concerns the interaction mechanism between the pair of molecules, no exponentially decreasing short range force, e.g., chemical bonds, can be responsible. Neither can it be a type of force with considerable relaxation time, as, e.g., Debye-Hueckel forces. Neither can it be a force

transmitted by the molecules of the medium in between the pair of molecules, because Brownian motion of the medium would jam the interaction. Thermally excited molecular vibrations are accompanied by vibrating electrical dipole moments which interact instantaneously for all practical purposes at short distances between the pair.

Let us first mention how specific attraction cannot be produced. Consider, for simplicity, a pair of harmonic vibrators, e.g., two negatively charged mass points which can vibrate about two positive centers only along the line connecting them with displacements s_I, s_{II}. R is the distance between the centers. The partition function in *classical* statistical mechanics

$$Z = \int \int \exp(-T/kT)dp_{sI}\, dp_{sII} \int \int \exp(-V/kT)ds_I\, ds_{II} \quad (1)$$

$$T + V = \frac{1}{2}(m_I^{-1}p_{sI}^2 + m_{II}^{-1}p_{sII}^2) + \frac{1}{2}(k_I^2 s_I^2 + k_{II}^2 s_{II}^2) - 2e^2 R^{-3}s_I s_{II}$$

will contain the masses m_I, m_{II} only through the first double integral, that is as factors in Z. Thus, the attractive force $-kT\partial \log Z/\partial R$ is independent of the masses, that is, independent of whether the frequencies of the pair are identical or not. The same holds if each molecule is represented by a set of vibrators, harmonic or anharmonic.

Quantum mechanical resonance of a pair of *simple harmonic* vibrators will not lead to a specific attraction either. Let the s direction be along the line connecting the two molecules. The molecules' orientation with respect to the axes may be described by stating that the components of the electric dipole moments of the two vibrators I, II are

$$\mu_{xI}q_I,\ \mu_{yI}q_I,\ \mu_{sI}q_I,\ \mu_{xII}q_{II},\ \mu_{yII}q_{II},\ \mu_{sII}q_{II},$$

where $q_I = m^{1/2}s_I$ if orientated in s direction. We consider first the interaction for a given, fixed, mutual orientation of the molecules; the ratios of the μ's are regarded as parameters which describe orientation effects to be discussed later. With the Hamiltonian ($\kappa_I = k_I/m^{1/2}$)

$$H = \frac{1}{2}(\dot{q}_I^2 + \dot{q}_{II}^2) + \frac{1}{2}(\kappa_I^2 q_I^2 + \kappa_{II}^2 q_{II}^2) +$$
$$R^{-3}[\mu_{xI}\mu_{xII} + \mu_{yI}\mu_{yII} - 2\mu_{sI}\mu_{sII}]q_I q_{II} \quad (2)$$

we find for the classical frequencies ω_+ (inphase mode) and ω_- (antiphase mode)

$$\left.\begin{array}{l} \omega_\pm^2 = \frac{1}{2}(\kappa_I^2 + \kappa_{II}^2)(1 \pm \epsilon); \\ \epsilon = \{(\kappa_I^2 - \kappa_{II}^2)^2 + 4R^{-6}[\mu\mu]^2\}^{1/2}/(\kappa_I^2 + \kappa_{II}^2) \end{array}\right\} \quad (3)$$

where $[\mu\mu]$ is the square bracket of equation (2) and the signature of the square root in the expression for ϵ has to be taken equal to the signature of $[\mu\mu]$. Figure 1 shows this well-known behavior of the proper frequencies, the vertical lines representing the influence of the coupling. Note the lack of sharpness of this resonance effect: Far away from $\kappa_I = \kappa_{II}$ we still have a strong influence of resonance.[2]

$$v \propto d_0 \sqrt[3]{(\rho' - \rho)^2 {}_{\rho\mu}^{-1-1}}.$$ (6)

In this expression the linear dimension d_0 is given by

$$d_0 = d - \xi d_s$$ (7)

where d_s, the so-called critical diameter, is given, according to Allen, by

$$d_s = \sqrt[3]{\frac{9\mu^2}{2g\rho(\rho' - \rho)}}$$ (8)

and ξ is a constant depending on the shape of the falling body.

Little data exists on the rate of passive sinking of freshwater planktonic organisms. Such information as has been recorded suggests that in the cases of bacteria, protozoa, unicellular algae and nearly all rotifers $N \lesssim 0.5$ and the rate of falling is therefore likely to be directly proportional to the square of the appropriate linear diameter, and to the density difference, and inversely proportional to the viscosity. For somewhat larger organisms the less well-known relationship derived by Allen may be expected to hold, the velocity being directly proportional to the appropriate linear diameter less a small correction, to the two-thirds power of the density difference and inversely to the cube root of the viscosity and density of the medium. It is unlikely that Newton's law is of any interest in the study of the plankton. Bowkiewicz[2] who alone has attempted to go beyond Stokes' law in the study of sinking speeds unfortunately made two mistakes, one in the interpretation of his data and one in supposing that the reciprocal of the square root of the viscosity entered into Newton's formulation.

We have determined the rate of falling of a number of specimens of *Daphnia* sp. 1[3] of various sizes from Bantam Lake, Conn., at temperatures between 21.7° and 27.2°C., corresponding to viscosities between 0.0085 and 0.0097 poise. All specimens were narcotized with ethyl urethane and had open antennae; they sank at various angles, the long axis being inclined with the head upward at angles from about 10° to about 80° above the horizon. The experiments were conducted in a cylinder of internal diameter 7.8 cms. For a sphere of diameter d falling in such a cylinder the velocity must be raised by a factor of $\left(1 + \dfrac{2.1d}{7.8}\right)$ according to Ladenburg's correction for the effect of the walls of the vessel. In the case of our smallest animal if d be taken as the length of the body the correction would amount to multiplication by 1.016, in the case of the largest to multiplication by 1.048. Actually a sphere of diameter d has a larger volume than an animal of length d and it is practically certain that such a correction would be excessive.

In figure 1 all the data, uncorrected for the effect of the walls, have been plotted against body length (exclusive of tail spine) on a double logarithmic grid. The maximum effect of the size of the vessel is indicated by the single open circle which is the corrected position of the point immediately below it, and which almost certainly represents an overcorrection. Although there is a fair amount of irregularity, it is evident that the points tend to fall along the line of slope 2, corresponding to direct proportionality between velocity and the square of the length. It is probable that most of the irregularity is due to differences in the density dependent on differences
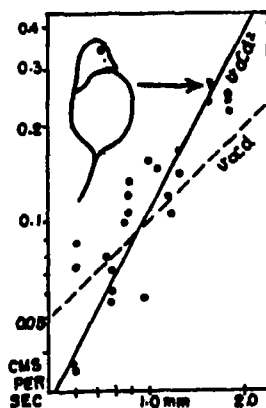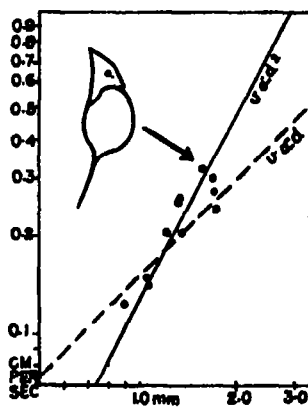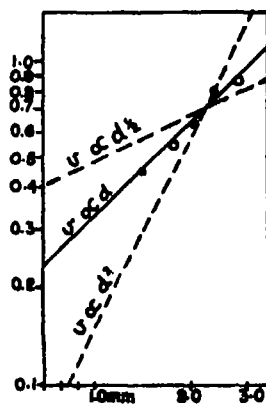

FIGURE 1

Relation of sinking velocity of *Daphnia* sp. 1 to length (tail spine excluded), the open circle indicates the maximum effect of Ladenburg's correction. Inset shows an experimental animal in lateral view.

FIGURE 2

Relation of sinking velocity of *Daphnia* sp. 2 to length (tail spine excluded). Inset of an experimental animal in lateral view.

FIGURE 3

Relation of sinking velocity of *Daphnia pulex* to length, from the data of Eyden;[4] solid circles measured individuals, open circles means of groups.

in the nutritive condition of the animal. In so far as the rather uncertain correction for the effect of the walls of the vessel is significant, it would raise the values of the velocity for the larger animals a little, improving the fit at the upper end of the curve to a very slight extent.

A few determinations of sinking speeds for a series of animals of almost identical lengths (0.86–0.89 mm.) were made at different temperatures and so at different viscosities. The results are plotted in the lower part of figure 4, from which it can be seen that in spite of the meagerness of the data there is an evident inverse relationship with the first power of the viscosity.

We chose interacting *harmonic* vibrators because the quantum levels can be simply expressed in terms of ω_+ and ω_-. The dipole-dipole interaction between the pair results in level shifts, upwards and downwards, which are strongest if the two vibrators have identical frequencies $\kappa_I = \kappa_{II}$. But the principal term of $\partial \log Z/\partial R$ turns out to be independent of whether the vibrators have identical frequencies or not; anharmonicity of the vibrators will not change this fact. To show this let us form the partition function Z for the set of levels shown in figure 2.[6] We see immediately from this figure that n_+ predominates over n_- among the thermally accessible quantum states, i.e., we get attraction. A simple calculation gives

$$\log Z = -\{ {}^{1}/_{2}\hbar\omega_+/kT + \log (1 - \exp (-\hbar\omega_+/kT)) + {}^{1}/_{2}\hbar\omega_-/kT + \log (1 - \exp (-\hbar\omega_-/kT))\}. \quad (4)$$

In the classical limit $\hbar\omega_\pm/kT \ll 1$

$$\partial \log Z/\partial R \rightarrow {}^{1}/_{2}(1 - \epsilon^2)^{-1}\partial \epsilon^2/\partial R \quad (5)$$

whereas in the upper limit $\hbar\omega_\pm/kT \gg 1$

$$\partial \log Z/\partial R \rightarrow \frac{1}{8}(1 + \frac{5}{8}\epsilon^2)\left(\frac{\kappa_I^2 + \kappa_{II}^2}{2}\right)^{1/2}\frac{\hbar}{kT}\frac{\partial \epsilon^2}{\partial R} \quad (6)$$

The expression (3) for ϵ shows that the term ($\partial \epsilon^2/\partial R$) does not depend upon whether $\kappa_I^2 - \kappa_{II}^2$ is small or not. This demonstrates the insensitivity of attraction of a pair of simple vibrators with respect to their frequencies being identical or not. The same holds if we use higher approximation in evaluating (6).

Quite a new situation arises if we consider quantum mechanical resonance between a pair of molecules, each of them having a *number of vibrational modes;* and if we take account of the anharmonic terms in the potential energy of vibration of each of the molecules. Let us consider first one molecule as a set of vibrators *which are coupled because of anharmonic terms.* Although we can get rid of quadratic cross-product terms by introducing "normal coordinates," the presence of anharmonic terms means that we certainly cannot abolish cubic and higher cross-product terms in the potential energy, and those will cause coupling between the "modes" of a molecule. In a complicated molecule there will be many "modes" which are almost commensurable (that is, much accidental degeneracy and resonance) which causes strong level shifts. In correspondence terms, certain phase relationships between the classical phases of the almost commensurable modes of one molecule will have lower energy and, therefore, because of the Boltzmann factor, be statistically preferred— the more preferred, the stronger the anharmonicity. This quantum effect depends on h/T.[7]

For simplicity, consider first the case of strict phase locking between the modes of each one of the molecules, instead of just phase preferences. This case is approached if there are strong anharmonicities and if there are many commensurabilities in the molecule. Let us then consider a pair of anharmonic molecules, each with phase-interlocked vibrations and the pair coupled by ordinary electric dipole-dipole interaction. If the lowest of the commensurable modes of molecule I is inphase with the lowest of II (regarding their z components), and if the molecules are identical, the other commensurable modes will be pairwise inphase also, which amounts to a strong negative interaction energy and thus a big contribution toward
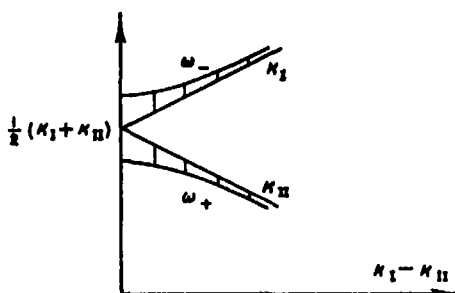
FIGURE 1

Inphase and antiphase frequencies in the vicinity of resonance.
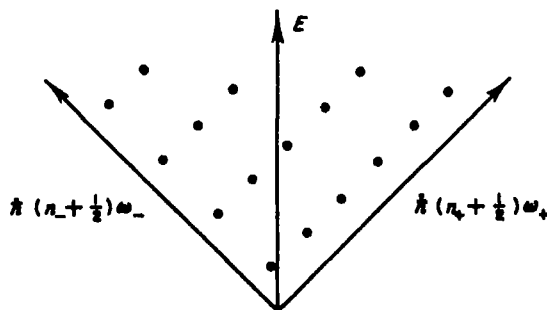
FIGURE 2

Example of thermally accessible quantum states.

the partition function. If the lowest modes of the two molecules are antiphase, the vibrations contribute little to the partition function. Such a pair of molecules behaves like a pair of vibrators with very large vibrational amplitudes; we may apply again the figure 2 as representing the thermally accessible quantum states of the pair of molecules only that higher statistical weights will have to be attributed to higher quantum states. More precisely: A state $n_+ = 4$, $n_- = 1$ which indicates a strong

excitation of the inphase mode and a weak excitation of the other mode will, in this picture, have the same statistical weight as a state $n_+ = 1$, n_- $= 4$; the statistical weights will very strongly increase with higher quantum numbers of this representative pair of large amplitude vibrators. Thermal accessibility on the other hand, can be represented by a horizontal line $E = $ const., limiting the region of accessible states. In consequence, the thermal excitation of the inphase mode will be overwhelming compared with that of the antiphase mode, i.e., the number of thermally excited states in which n_+ dominates over n_- is much larger than those in which $n_- > n_+$. The net effect of the contributions of all quantum states amounts to a decrease of the free energy, that is an attraction.

If we consider phase preferences instead of strict phase locking inside the molecules, we will get a similar effect but of smaller order. In the classical limit $h/T \to 0$, resonance effects and specific attraction disappear. Clearly this attraction is specific with respect to the two molecules being structurally and therefore vibrationally identical, as the phase preferences within one molecule will match those within the other only provided that the molecules are identical and properly orientated.

Equation (2) tells us about orientation effects. Consider two identical molecules in the same mutual orientation as are two identical closed dictionaries which lie right on top of each other, each dictionary's front cover facing upward, but the bindings facing in opposite directions. Let the z axis in this illustration be vertical, i.e., in the direction connecting the centers of the two molecules (dictionaries). If each of the molecules vibrates *en bloc* with established phase relationships and if the pair of molecules vibrates in synchronism, we have a form of motion in which the z_I, z_{II} components of vibration all vibrate, e.g., inphase and therefore, all x_I, x_{II} components and all y_I, y_{II} components vibrate in antiphase. So all three components contribute an attractive interaction potential. Such a peculiar kind of mutual orientation is caused by the same effect as the attraction itself; the pair has lower energy if orientated in that way; Brownian motion will bring about this orientation.

The orientation effects in the interaction-term of (2) will cause a molecule with laevo configuration to have a strong interaction but with another laevo molecule; the same a dextro with a dextro, as a short consideration of various orientations readily shows. This accounts for the fact that a laevo structure always builds up a laevo structure, and that an admixture of some dextro will cause considerable trouble with respect to its acceptability.

After a pair of molecules has come close together, in the energetically most favorable orientation, the dipole field of the pair is altogether different from that of a single molecule which prevents the pairs field from matching the field of a third identical molecule, thereby failing to attract the third.

So triplets are not formed. Another pair may, however, be attracted; so we may expect formation of pairs, quadruplets, octuplets, etc.

We shall expect this specific attraction effect to occur on various scales, probably from small peptide rings up to larger constituent molecules of genes, to genes and gene-complexes and correspondingly for viruses.

A qualitative argumentation as this one is more easily carried through in terms of Bohr's formulation of quantum theory[3] and the correspondence principle rather than by straight quantum mechanics.

Because of the complications of straight-forward quantum mechanical calculations of a pair of identical molecules with commensurable modes, we simplify the model, and show that the above-mentioned effects exist. Anharmonicity strongly couples commensurable modes; quantum mechanically, anharmonicity brings about level splitting (level shifts) between various (e.g., symmetric or anti-symmetric) combinations of resonating ψ functions. These level shifts are the stronger, the stronger the anharmonicity, the closer the commensurability and the larger the number of resonating modes.

Interactions between "modes" in a molecule I exist only if there is some anharmonic perturbation function, e.g. $\lambda q_{1I}{}^2 q_{2I}$ in case of commensurability 1 to 2 between the modes, or other more complicated perturbation functions. Similarly for other commensurability ratios, and for the molecule II. The effects, discussed above in terms of the correspondence principle do, however, not much depend on the kind of coupling which is assumed to exist in between the modes of each one of the molecules. Therefore, in our simplified crude model we represent this situation by an interaction $\lambda q_{1I} q_{2I}$ and $\lambda q_{1II} q_{2II}$ of two exactly equal frequency harmonic pseudomodes inside each of the identical molecules I and II. We assume q_{2I} and q_{2I} parallel orientated and λ negative. As we look for a quantum effect we assume that, besides the groundlevel 0, only the next bunch of levels 1 is thermally excited. A detailed calculation showed that for the effect under consideration only first-order perturbation theory needs to be applied. We assume a simple perturbation function

$$H_1 = \lambda(q_{1I}q_{2I} + q_{1II}q_{2II}) + \mu(q_{1I}q_{1II} + q_{2I}q_{2II} + q_{1I}q_{2II} + q_{2I}q_{1II}) \quad (7)$$

where μ is an expression of the type

$$R^{-3}[\mu_{xI}\mu_{xII} + \mu_{yI}\mu_{yII} - 2\mu_{zI}\mu_{zII}]. \quad (8)$$

H_1 becomes diagonal with the eigenfunctions

$$\psi_{00} = \psi_0(q_{1I})\psi_0(q_{2I})\psi_0(q_{1II})\psi_0(q_{2II}) = (0000)$$
$$\psi_{10} = \frac{1}{2}\{(0100) + (1000) + (0001) + (0010)\}$$
$$\psi_{11} = \frac{1}{2}\{(0100) + (1000) - (0001) - (0010)\}$$
$$\psi_{12} = \frac{1}{2}\{(0100) - (1000) + (0001) - (0010)\} \quad (9)$$
$$\psi_{13} = \frac{1}{2}\{(0100) - (1000) - (0001) + (0010)\}$$

In order to show how dispersion forces fit into this scheme, we calculate the ground level up to second order perturbation terms (using perturbations due to levels up to the second bunch).

$$E_{00} = (4 - (\lambda^2 + 2\mu^2)/2\kappa^4)^{1}/_2\hbar\kappa$$
$$E_{10} = [6 + (\lambda + 2\mu)/\kappa^2]^{1}/_2\hbar\kappa$$
$$E_{11} = [6 + (\lambda - 2\mu)/\kappa^2]^{1}/_2\hbar\kappa \qquad (10)$$
$$E_{12} = [6 - \lambda/\kappa^2]^{1}/_2\hbar\kappa$$
$$E_{13} = [6 - \lambda/\kappa^2]^{1}/_2\hbar\kappa$$

This shows the statistical preference of the "inphase" mode ψ_{10} over the three other excited ψ_{11}, ψ_{12}, ψ_{13}. The negative of the attractive force becomes

$$kT\partial \log Z/\partial R = kTZ^{-1} (\partial Z/\partial\mu)(d\mu/dR) \sim kT \frac{d\mu}{dR} \exp \frac{2\hbar\kappa}{kT}.$$

$$\left[1 - 4 \exp\left(-\frac{\hbar\kappa}{kT}\right)\right] \cdot \frac{\partial}{\partial\mu}\left[\exp\left(-\frac{E_{00}}{kT}\right) + \exp\left(-\frac{E_{10}}{kT}\right) + \right.$$

$$\left.\exp\left(-\frac{E_{11}}{kT}\right) + \cdot + \cdot\right] \sim kT \frac{d}{dR}\left(\frac{\mu}{\kappa^2}\right).$$

$$\frac{\mu}{\kappa^2}\frac{\hbar\kappa}{kT}\left[1 - 4 \exp\left(-\frac{\hbar\kappa}{kT}\right) + 2 \frac{\hbar\kappa}{kT}\exp\left\{-\left(1 + \frac{1}{2}\frac{\lambda}{\kappa^2}\right)\frac{\hbar\kappa}{kT}\right\}\right] \quad (11)$$

This shows that the effect is a quantum effect and that it is proportional to R^{-7}, cf. (8). The effect also depends on the factor

$$\exp\left(-\frac{1}{2}\frac{\lambda}{\kappa^2}\frac{\hbar\kappa}{kT}\right) \qquad (12)$$

i.e., it depends exponentially on the strength $(-\lambda)$ of coupling in between the modes of each one of the molecules. The first and second term in the last square bracket in (11) originates from $\partial E_{\infty}/\partial\mu$ and represents London's dispersion forces. A similar calculation for a pair of molecules, each one containing n equal modes, shows a much stronger influence of λ of the same character; in a big molecule the factor corresponding to (12) will be large compared with one, it becomes $\exp\{-1/2(n-1)\lambda\kappa^{-2}\hbar\kappa/kT\}$.

In order to study the specificity of attraction in the case of our simplified model, we applied a small detuning δ, $(\kappa^2\delta^2 \gg \lambda^2)$ and calculated its influence on the λ term (12) of the force (11). The case $\kappa_{1I} = \kappa_{2I} = \kappa + \delta$, $\kappa_{1II} = \kappa_{2II} = \kappa - \delta$ brings, like (5) and (6), no change, i.e. again (11). The case of $\kappa_{1I} = \kappa_{1II} = \kappa + \delta$, $\kappa_{2I} = \kappa_{2II} = \kappa - \delta$ makes the term (12) disappear, so does the case $\kappa_{1I} = \kappa + \delta$, $\kappa_{1II} = \kappa - \delta$, $\kappa_{2I} = \kappa_{2II} = \kappa$, and also the case $\kappa_{1I} = \kappa + \delta$, $\kappa_{2I} = \kappa - \delta$, $\kappa_{1II} = \kappa_{2II} = \kappa$. In other words, the attraction is specific with respect to the latter three changes.

This is the effect of phase preferences on the attraction. Our former argumentations make it evident that in the case of commensurable rather than equal modes the effect of *identical* phase preferences in the pair of molecules which depends on the identity of the pair, is causing the specificity.

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INHERITANCE OF SEXUALITY IN CHLAMYDOMONAS REINHARDI*

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Moewus[1-3] has studied the inheritance of sexuality in haploid heterothallic (dioecious) algae where the zygote nucleus divides meiotically and where a germinating zygote produces four haploid cells in which two of the cells are of one sex and two of the opposite sex. He has analyzed sexuality of cells from germinating zygotes of five heterothallic species of *Chlamydomonas* and one heterothallic species of *Polytoma*. For all species investigated he finds exceptional cases in which all cells produced by a germinating zygote are homothallic (monoecious) instead of all being heterothallic. For zygotes produced by union of male and female cells of the same species he found that the percentage of exceptions ranged between 2.8 and 7.8. In germinating zygotes formed by a cross between *Chlamydomonas paradoxa* and *C. pseudoparadoxa* the percentage of exceptions was 11.7. Moewus holds that the genes for maleness and femaleness are not alleles, but are at different loci on homologous chromosomes, and that as a result of crossing-over one chromosome contains the genes for both sexes and the other chromosome lacks genes for sex. Daughter nuclei lacking genes for sex disintegrate; those with genes for both sexes persist and cells with such nuclei are homothallic.

Plus and minus (male and female) clones of several species of *Chlamy-domonas* have been isolated at Stanford University. These have been cultured in an immobile palmelloid condition on 0.1% Beijerincks solution plus 1.5% agar. When palmelloid cultures growing on agar are flooded with distilled water the cells develop flagella and become motile shortly after flooding. If motile cells from clones of opposite sex are mixed there is an immediate fusion in pairs to form zygotes. The resultant zygotes become thick-walled within a few days, and are fully ripened in about three weeks. Germination of ripe zygotes may be induced by placing them on nutrient agar.

Of the species isolated at Stanford, *C. Reinhardi* proved the most satisfactory for study of inheritance of sexuality. It has relatively large zygotes; germinates 22–26 hours after transfer to nutrient agar; and about 90% of the zygotes germinate. Single zygotes of *C. Reinhardi* were isolated on blocks of agar about 5 mm. square. Four blocks, each with a single zygote, were quadrately arranged 5 cm. from one another on nutrient agar in a Petri dish. At the end of 26 hours each of the small blocks was flooded with a large drop of water. This induced motility in daughter cells extruded from germinated zygotes and the cells swam about in the small puddle of water. Swimming continued until the motile cells became stranded on the agar surface because of absorption of the water by the agar. Each stranded cell, by division, developed into a colony that at the end of ten days contained hundreds of cells. When the colonies were large enough to be visible to the naked eye, each was fished out with a sterile needle and transferred to an agar slant. The sexuality of the four or eight clones obtained from each germinated zygote was then determined by flooding with distilled water and noting the sexual reaction of the cells made motile by flooding.

The sexual reaction was determined for each of the clones developed from individual cells liberated from 249 zygotes of *C. Reinhardi*. None of these clones proved to be homothallic. A few (17) isolates did give a homothallic reaction when tested for sexuality. However, when clones were reisolated from vegetative cells of these putative homothallics all proved to be heterothallic and not homothallic. From this it is evident that the homothallic reaction of the original isolation was due to a heterothallic colony being invaded by cells of opposite sex.

All clones proved to be heterothallic and, where four or eight clones were obtained from a zygote, with half the clones of one sex and half of the opposite sex.

In view of the percentage of crossing-over of genes for sex reported by Moewus for five species of *Chlamydomonas* it is reasonable to expect that at least one case would have been found for *C. Reinhardi*. Since this has not been found in *C. Reinhardi* it is very probable that in this species the

genes for sex are at the same locus and not at different loci as Moewus finds in the species he investigated.

For inheritance of sex, as well as for inheritance of other characters, Moewus always reports obtaining four clones from a zygote. His papers do not show whether he always obtained four clones or whether he discarded material from zygotes where he obtained fewer or more than four clones. Germinating zygotes of *C. Reinhardi* may produce eight instead of four cells, and the number of zygotes producing eight cells is greater in old than in recently ripened ones. Although there is always a liberation of four or eight cells, there is not always a development of four or of eight colonies. For example, in a random selection of 100 zygotes recorded as liberating four cells, 83 had a development of four colonies, 11 a development of three colonies, and 6 a development of two colonies. The reason why certain liberated cells fail to divide and develop into colonies is as yet undetermined, but it is not correlated with sexuality. When the three colonies developed from a single zygote are tested for sexuality two are always of one sex and the third is of the opposite sex. When there is a development of but two colonies the two may be of the same sex, and either plus or minus, or one colony may be plus and the other minus.

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THE HOMOLOGY STRUCTURE OF SPHERE BUNDLES

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1. Since a fiber bundle is a generalization of a product space, it is natural to expect some relations between the homology groups of the bundle, the base space, and the fiber. The simplest relation is between the Euler characteristics, the characteristic of the bundle being the product of the characteristics of the base space and the fiber. For sphere bundles the first comprehensive result was obtained by Gysin.[1] Recently, Steenrod[2] gave a new derivation of the Gysin results. On the other hand, results have been announced by Hirsch[3] and Leray[4] for more general types of bundles.

In the works of both Gysin and Steenrod the base space is assumed to be an orientable manifold. The main purpose of our work is to extend

this result to the case that the base space is a complex and to furnish a simpler and more direct method of approach which can be applied to more general fiber bundles. The principal theorem is a statement that a certain sequence of homomorphisms of cohomology (or homology) groups is exact. It seems to us that this theorem includes all known results about the cohomology structure of sphere bundles. As a basis of our treatment we make use of the axiomatic homology theory of Eilenberg and Steenrod.[5]

In §2 we define the type of bundle to be studied. In §3 we state the main results. Some applications of these results are given in §4, including a proof of Gysin's main theorem and a derivation of some relations between the Betti numbers of the bundle, the base space and the fiber. An indication of the methods used is given in §5.

2. Let K denote a finite connected simplicial complex of dimension n. Let $B = |K|$ be the space of K, and let X be a topological space. A continuous map $f:X \to B$ is called a *fibering of X into d-spheres over B* if for each simplex s of K there exists a homeomorphism

$$\varphi_s:|s| \times S^d \approx f^{-1}(|s|),$$

where S^d denotes the d-dimensional sphere, such that:

(a) $f\varphi_s(y, z) = y$ for $y \in |s|$, $z \in S^d$.

(b) If s is a face of s', then

$$g_{ss'}(y):S^d \approx S^d$$

defined by

$$\varphi_s(y, g_{ss'}(y)(z)) = \varphi_{s'}(y, z),   \text{for } y \in |s|, z \in S^d$$

is a continuous mapping of $|s|$ into the group of homeomorphisms of S^d of degree $+1$.

X is called the *bundle*, and B is called the *base space* of the bundle. The mapping f is called the *projection* of the bundle. The projection induces homomorphisms

$$f^*:H^p(B) \to H^p(X)$$

of the cohomology groups of B into those of X over any coefficient group. In the following the coefficient group is arbitrary but fixed for all cohomology groups under consideration.

3. Using the bundle structure two homomorphisms will be defined. The first homomorphism maps the cohomology groups of X into those of B and lowers dimension by d. It is denoted by

$$\Phi:H^p(X) \to H^{p-d}(B).$$

This homomorphism has been considered by Gysin and Lichnerowicz[6] in the case when the base space is an orientable manifold.

The second homomorphism maps cohomology groups of B into cohomology groups of B and raises dimension by $d + 1$. It will be denoted by

$$\Psi : H^p(B) \rightarrow H^{p+d+1}(B).$$

These two homomorphisms together with f^* form an infinite sequence

$$\ldots \xrightarrow{\Psi} H^p(B) \xrightarrow{f^*} H^p(X) \xrightarrow{\Phi} H^{p-d}(B) \xrightarrow{\Psi} H^{p+1}(B) \xrightarrow{f^*} \ldots$$

called the *cohomology sequence of the bundle*.

The main result is embodied in the following theorem:

THEOREM 3.1. *The cohomology sequence of a sphere bundle is exact.*

This theorem shows that if the homomorphism Ψ can be determined in B then the cohomology groups of X are group extensions of groups defined in B by other groups defined in B. This shows the significance of characterizing the homomorphism Ψ, which can be described as follows:

Any sphere bundle gives rise in a natural way to a unique $(d + 1)$-dimensional cohomology class of B with integer coefficients.[7] This class is called the *characteristic class* of the bundle and will be denoted by Ω. It is the obstruction to the construction of a cross-section over the $(d + 1)$-dimensional skeleton of B. Using the natural pairing of the integers and the abelian coefficient group to the coefficient group, the cup products $u \cup \Omega$ and $\Omega \cup u$ are defined for $u \in H^p(B)$ and are elements of $H^{p+d+1}(B)$. The order of multiplication is immaterial. For odd d this follows simply from the commutation rule of the cup product. For even d it can be shown that $2\Omega = 0$, so that the two products are again equal. Then we have the theorem:

THEOREM 3.2. *For any $u \in H^p(B)$,*

$$\Psi(u) = u \cup \Omega = \Omega \cup u.$$

Naturally there exist dual theorems for the homology groups. In particular, we define homomorphisms

$$f_* : H_p(X) \rightarrow H_p(B),$$

$$\Phi' : H_p(B) \rightarrow H_{p+d}(X),$$

$$\Psi' : H_p(B) \rightarrow H_{p-d-1}(B).$$

These homomorphisms form an infinite sequence,

$$\ldots \xleftarrow{\Psi'} H_p(B) \xleftarrow{f_*} H_p(X) \xleftarrow{\Phi'} H_{p-d}(B) \xleftarrow{\Psi'} H_{p+1}(B) \xleftarrow{f_*} \ldots,$$

called the *homology sequence* of the bundle. The results for the case of homology are summarized in the following theorem:

THEOREM 3.3. *The homology sequence of a sphere bundle is exact. Moreover, if Ω denotes the characteristic cohomology class of the bundle, the homomorphism*

$$\Psi' : H_p(B) \to H_{p-d-1}(B)$$

satisfies the equation

$$\Psi'(z) = \Omega \cap z, \qquad for \ z \ \epsilon \ H_p(B),$$

4. Gysin's isomorphism theorem is contained in Theorem 3.3, for let $K_p(B)$ be the subgroup of $H_p(B)$ consisting of the kernel of Φ'. Then exactness of the sequence implies that Ψ' induces an isomorphism

$$\Psi : H_{p+d+1}(B)/f_* H_{p+d+1}(X) \approx K_p(B).$$

The inverse of this isomorphism is the isomorphism denoted by H by Gysin.

A slightly different isomorphism is obtained for cohomology if we define $K^p(B)$ to be the subgroup of $H^p(B)$ consisting of the kernel of Ψ. Then Φ induces isomorphisms

$$\bar{\Phi} : H^p(X)/f^* H^p(B) \approx K^{p-d}(B).$$

The inverse of $\bar{\Phi}$ is Steenrod's functional cup product.[2] More precisely, if $u \ \epsilon \ H^{p-d}(B)$ such that $u \cup \Omega = 0$, then $u \underset{f}{\cup} \Omega$ is defined and belongs to $H^p(X)/f^* H^p(B)$. Then we have

$$\Phi \ (u \cup \Omega) = u.$$

As a second application we study the influence of the relative position of the fiber in the bundle on the homology structure of the bundle. If S^d denotes a fiber, the inclusion map $i : S^d \subset X$ induces homomorphisms

$$i_* : H_d(S^d) \to H_d(X)$$

$$i^* : H^d(X) \to H^d(S^d).$$

The fiber is said to *bound in the bundle* ($S^d \sim 0$) relative to a coefficient group G if

$$i_* H_d(S^d) = 0$$

where $H_d(S^d)$ is taken over G. In terms of cohomology this condition is known to be equivalent to

$$i^* H^d(X) = 0,$$

$H^d(X)$ being taken over a group dual to G.

To avoid cumbersome statements we assume in the rest of this section that the coefficient group for homology and cohomology is a field.

The groups $H^0(B)$ and $H^d(S^d)$ are both isomorphic to the coefficient field and hence to each other. An isomorphism

$$\nu : H^0(B) \approx H^d(S^d)$$

between them can be found such that

$$\nu \Phi = i^*.$$

Therefore, if $S^d \sim 0$, then $K^0(B) = 0$. This in turn implies that $\Omega \neq 0$ and $k \Omega \neq 0$ for any integer k. In particular, if d is even, then $2 \Omega = 0$ so S^d does not bound in X.

If, conversely, S^d does not bound in X, the argument can be reversed, so we see that $k \Omega = 0$ for some integer k. It follows then from the cohomology sequence that there is an isomorphism between $H^p(X)$ and $H^p(B \times S^d)$.

Finally, we shall derive some relations between the Betti numbers of X and B, which were obtained by Gysin, Hirsch, and Leray. Let $\rho^p(X)$ and $\rho^p(B)$ be the pth Betti numbers of X and B and $\rho_0^p(B)$ the dimension of the kernel of Ψ in $H^p(B)$. Then we have, from the cohomology sequence of the bundle,

$$\rho^p(X) = \rho^p(B) - \rho^{p-d-1}(B) + \rho_0^{p-d-1}(B) + \rho_0^{p-d}(B). \qquad (4.1)$$

Let $X(t)$, $B(t)$, $S^d(t)$ be the Poincaré polynomials of X, B, S^d, respectively, and let

$$P(t) = \sum_{p \geq d} (\rho^{p-d}(B) - \rho_0^{p-d}(B)) t^p,$$

so that $P(t)$ has non-negative coefficients. It is easily verified by using (4.1) that

$$X(t) = S^d(t)B(t) - (1 + t)P(t),$$

and that

$$[1 - t(S^d(t) - 1)]B(t) \leq X(t) \leq S^d(t)B(t),$$

where an inequality between polynomials means a set of inequalities between their corresponding coefficients.

5. We shall indicate in this section the method used in proving the theorems stated in §3.

Let $B^p = |K^p|$ be the space of the p-dimensional skeleton of K. Let $X_p = f^{-1}(B^p)$. Then

$$X = X_n \supset \ldots \supset X_p \supset X_{p-1} \supset \ldots \supset X_0 \supset X_{-1} = 0.$$

Using the fact that $f^{-1}(|s|)$ is homeomorphic to $|s| \times S^d$ for each simplex s of K, it is easy to prove the following

LEMMA 5.1. *Let* $f_p:(X_p, X_{p-1}) \to (B^p, B^{p-1})$ *be the map defined by* f. *Then*

$$f_p^*:H^p(B^p, B^{p-1}) \approx H^p(X_p, X_{p-1})$$

is an isomorphism onto. Also there is an isomorphism

$$\lambda_p:H^{p-d}(B^{p-d}, B^{p-d-1}) \approx H^p(X_{p-d}, X_{p-d-1}),$$

while

$$H^q(X_p, X_{p-1}) = \{0\}, \text{ if } q \neq p, p + d.$$

The group of cochains of K, $C^p(K)$, is defined by

$$C^p(K) = H^p(B^p, B^{p-1}),$$

so the lemma shows that $H^q(X_p, X_{p-1})$ is either the trivial group or is isomorphic to a group of cochains of K.

The following diagram will be referred to as the main diagram

In the main diagram δ_p and $\bar{\delta}_p$ are coboundary operators and α_p, β_p, γ_p, ρ_p, σ_p, τ_p are induced by inclusion mappings. Using the exactness axiom of cohomology theory and (5.4) the following can be proved.

LEMMA 5.5. *For any* $p \geqq 0$, *the sequence of homomorphisms* α_p, β_p, δ_p, ρ_{p+1}, γ_{p+1}, $\bar{\delta}_{p+1}$, σ_{p+2}, τ_{p+2} *is exact.*

The fact that in defining the bundle, $g_{ss'}(y)$ is required to be a homeomorphism of S^d of degree $+1$ furnishes the following results.

LEMMA 5.6. *Commutativity holds in the diagram*

$$H^p(X_p, X_{p-1}) \xrightarrow{\delta_p \rho_p} H^{p+1}(X_{p+1}, X_p)$$

$$f_{p^*}\Big\uparrow \qquad\qquad \Big\uparrow f_{p+1^*}$$

$$C^p(K) \xrightarrow{\delta} C^{p+1}(K).$$

LEMMA 5.7. *Commutativity holds in the diagram*

$$H^p(X_{p-d}, X_{p-d-1}) \xrightarrow{\delta_p \rho_p} H^{p+1}(X_{p-d+1}, X_{p-d})$$

$$\lambda_p\Big\uparrow \qquad\qquad \Big\uparrow \lambda_{p+1}$$

$$C^{p-d}(K) \xrightarrow{\delta} C^{p-d+1}(K)$$

We now proceed to construct the homomorphisms used in forming the cohomology sequence of the bundle from the main diagram.

To compute

$$f^* : H^p(B) \to H^p(K)$$

from the main diagram, let $u \in H^p(B)$. Let $c \in C^p(K)$ represent u. Then $\delta c = 0$, so that $\delta_p \rho_p f_p{}^*(c) = 0$. Hence there exists $v \in H^p(X)$ such that

$$\beta_p(v) = \rho_p f_p{}^*(c).$$

It is easy to see that $f^*(u) = v$.

To define the homomorphism

$$\Phi : H^p(X) \to H^{p-d}(B),$$

let $u \in H^p(X)$. Then $\gamma_p \beta_p(u) \in H^p(X_{p-d})$. Since $\tau_p(\gamma_p \beta_p(u)) = 0$, there is $v \in H^p(X_{p-d}, X_{p-d-1})$ such that

$$\sigma_p(v) = \gamma_p \beta_p(u).$$

By (5.3) there is $c \in C^{p-d}(K)$ such that

$$\lambda_p(c) = v.$$

Then

$$\lambda_{p+1}\delta(c) = \delta_p \sigma_p \lambda_p(c) = \bar\delta_p \sigma_p(v) = \bar\delta_p \gamma_p \beta_p(u) = 0,$$

so $\delta(c) = 0$. It follows that c is a cocycle of K and determines a cohomology class of B. This class depends only on u and is defined to be $\Phi(u)$.

To define

$$\Psi : H^{p-d}(B) \to H^{p+1}(B),$$

let $u \in H^{p-d}(B)$. Choose $c \in C^{p-d}(K)$ to represent u. Then $\delta c = 0$, so $\delta_p \sigma_p \lambda_p(c) = 0$. Hence there is $v \in H^p(X_p)$ such that

$$\gamma_p(v) = \sigma_p \lambda_p(c).$$

Now $\delta_p(v) = \bar{v} \epsilon H^{p+1}(X_{p+1}, X_p)$. Let $\bar{c} \epsilon C^{p+1}(K)$ such that $f_{p+1}^*(\bar{c}) = \bar{v}$. Since

$$\delta_{p+1}\rho_{p+1}(\bar{v}) = \delta_{p+1}\rho_{p+1}\delta_p(v) = 0,$$

it follows that $\delta(\bar{c}) = 0$. The cohomology class in B to which c belongs depends only on u and is defined to be $\Psi(u)$.

Having defined the homomorphisms f^*, Φ, Ψ, Theorem 3.1 follows immediately from Lemma 5.5.

If the integers are used as coefficients, let $\omega \epsilon H^0(B)$ be the unit cohomology class in B. It is easy to show that the characteristic class Ω satisfies the equation

$$\Omega = \Psi(\omega).$$

The following is a brief sketch of the proof of Theorem 3.2. We consider $X \times B$ as a bundle over $B \times B$. Let Ψ_1 be the homomorphism Ψ defined for this bundle. Then it can be shown that

$$\Psi_1(\alpha \times \beta) = \Psi(\alpha) \times \beta, \qquad \text{for } \alpha \epsilon H^p(B), \beta \epsilon H^q(B).$$

Let $d:B \to B \times B$ be the diagonal map. The induced bundle by d over B is equivalent to the given bundle. It follows from this that

$$d^*\Psi_1 = \Psi d^*.$$

Then

$$d^*\Psi_1(\omega \times u) = \Psi(\omega \cup u) = \Psi(u).$$

But

$$d^*\Psi_1(\omega \times u) = d^*(\Psi(\omega) \times u) = \Psi(\omega) \cup u = \Omega \cup u.$$

Added in proof March 27, 1950. While this was in press an article by Thom appeared in the *C. R. Paris* of January 30, 1950 which essentially contains our theorems 3.1 and 3.2.

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ELIMINATION OF RANDOMIZATION IN CERTAIN PROBLEMS OF STATISTICS AND OF THE THEORY OF GAMES

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1. The main aim of this note is to show that mixed strategies may be eliminated from statistical decision rules based on the observation of random variables with continuous distribution functions, and from games of a similar structure.

After stating a general measure-theoretic result in 2. we deduce in 3. and 4. some results on games. In 5. and 6. we give some results on decision problems. Our methods lead to considerably more general results than those presented here (in particular they can be applied to what may be termed sequential games). The selection of results presented here was motivated by our interest in statistical problems. Proofs and a more complete study of the subject will be published elsewhere.

2. Let $\{x\} = X$ be any space and $\{S\} = \mathcal{S}$ a Borel field of subsets of X. Let $\mu_k(S)$ $(k = 1, 2, \ldots, p)$ be a finite number of real-valued, bounded and countably additive set functions defined for all $S \in \mathcal{S}$. The fundamental measure theoretic result mentioned in the introduction is the following:

THEOREM 1. *Let $\eta_j(x)$ $(j = 1, 2, \ldots, n)$ be real non-negative $\mathcal{S}$-measurable functions satisfying*

$$\sum_{j=1}^{n} \eta_j(x) = 1 \qquad \text{for all } x \in X. \tag{1}$$

Then, if the set functions $\mu_k(S)$ $(k = 1, 2, \ldots, p)$ are non-atomic,[1] there exists a decomposition of X into n disjoint subsets $S_1, \ldots, S_n$ belonging to $\mathcal{S}$ which have the property that

$$\int_x \eta_j(x)d\mu_k(x) = \mu_k(S_j) \qquad (j = 1, 2, \ldots, n; \ k = 1, 2, \ldots, p). \tag{2}$$

The proof of this theorem makes use of a result of A. Liapounoff[2] on the ranges of vector measures and yields, incidentally, an extension of his result. The non-atomicity requirement is indispensable. It is this assumption that is responsible for the possibility to disregard mixed strategies in the games treated here, as opposed to the finite games originally considered by J. von Neumann.[3]

3. Consider now a two-person zero-sum game where player I has a finite number of pure strategies i $(i = 1, 2, \ldots, m)$ while the pure strategies of player II consist of $\mathcal{S}$-measurable functions $l(x)$ defined over X and

assuming only the values $1, 2, \ldots, n$.   The outcome of the game when the above pure strategies are adopted is given by

$$K(i, l(x)) = \sum_{j=1}^{n} \mu_{ij}(S_j),\tag{3}$$

where S_j is the set where $l(x)$ assumes the value j and the $\mu_{ij}(S)$ $(i = 1, \ldots, m; \ j = 1, \ldots, n)$ are real-valued, bounded, countably additive set functions defined for $S \in \mathcal{S}$.

The general, mixed or pure, strategies of I may be represented by vectors $\xi = (\xi_1, \ldots, \xi_m)$ of non-negative components adding up to one, while the general strategy of II may be written as a vector function $\eta(x) = (\eta_1(x), \ldots, \eta_n(x))$ the components of which are non-negative S-measurable functions satisfying (1).   When these strategies are adopted the outcome of the game is

$$K(\xi, \eta(x)) = \sum_{i=1}^{m} \xi_i \sum_{j=1}^{n} \int_X \eta_j(x) d\mu_{ij}(x).$$

A pure strategy of II may be also represented by a vector $\eta(x)$, only now the additional assumption is made that all components are either zero or one for all x.   We easily deduce from Theorem 1 the following:

THEOREM 2.   *If the set functions $\mu_{ij}(S)$ $(i = 1, \ldots, m; \ j = 1, \ldots, n)$ are non-atomic, then to every strategy $\eta(x)$ of player II there exists a pure strategy $\eta^*(x)$ equivalent to it, i.e., such that $K(\xi, \eta(x)) = K(\xi, \eta^*(x))$ for all strategies ξ of player I.*

In most cases the functions $\mu_{ij}(S)$ are given by $\mu_{ij}(S) = \int_S a_{ij}(x) d\mu_i(x)$, where the $\mu_i(S)$ $(i = 1, \ldots, m)$ have the properties described above and the $a_{ij}(x)$ $(j = 1, \ldots, n)$ are S-measurable real-valued functions integrable with respect to $\mu_i(S)$.   The special case when the functions $a_{ij}(x)$ reduce to constants is particularly important.   For this case we have

THEOREM 3.   *If in the preceding theorem $\mu_{ij}(S) = a_{ij}\mu_i(S)$ then whatever $\eta(x)$ there exists a pure strategy $\eta^*(x)$ equivalent to it simultaneously for all choices of the constants a_{ij}.*

4.   We now return to Theorem 1, but instead of considering the case where there existed a finite number of functions $\mu_k(S)$ we consider the case when k varies in some abstract space K.   Let us introduce a metric g into the space K by putting $g(k, k') = \sup |\mu_k(S) - \mu_{k'}(S)|$, the sup being taken with respect to $S \in \mathcal{S}$.   It is clear that if all other conditions of Theorem 1 are satisfied and the space M, with the metric g, is conditionally compact then, given $\epsilon > 0$, one may find a decomposition of X into disjoint $S_j \in \mathcal{S}$ such that

$$| \int_X \eta_j(x) d\mu_k(x) - \mu_k(S_j) | < \epsilon \qquad (j = 1, \ldots, n; \ k \in K).$$

We now consider games of the kind described in 3. except that we drop the restriction that i vary over a finite set and allow it to vary over an arbitrary space I. The outcome when pure strategies are adopted is again given by (3), while generally it is given by

$$K(\xi, \eta(x)) = \int_I \left\{ \sum_{j=1}^n \int_x \eta_j(x) d\mu_{ij}(x) \right\} d\xi_i,$$

$\xi = \xi_i$ being a probability distribution over I representing the first player's strategy.

From the preceding application of Theorem 1 we have:

THEOREM 4. *If the set functions* $\mu_{ij}(S)$ ($i \in I$, $j = 1, \ldots, n$) *are non-atomic and* I *is conditionally compact according to the metric*

$$g(i, i') = \max_{j=1,\ldots,n} \sup_{S \in \mathcal{S}} |\mu_{ij}(S) - \mu_{i'j}(S)| \qquad (i, i' \in I)$$

then to every $\epsilon > 0$ *and every strategy* $\eta(x)$ *of the second player, there exists a pure strategy* $\eta^*(x)$ *of this player satisfying* $|K(\xi, \eta^*(x)) - K(\xi, \eta(x))| < \epsilon$ *for all strategies* ξ *of the first player.*

A theorem recently proved by R. Bellman and D. Blackwell[4] is a very special case of this result. Theorem 4 can evidently be extended to include the case when j also has an infinite range.

5. A rather extensive class of fixed sample size decision problems in statistics may be described as follows.[5] A random variable x with range in a t-dimensional Euclidean space X is distributed according to an unknown one of a finite number m of distributions, the possible (cumulative) distribution functions being $F_i(x)$ ($i = 1, \ldots, m$). An observation (this means, in general, a sample of size t) is made and according to the observed value x the statistician may adopt any one of n decisions j. (In many cases $m = n$ and the statistician has to decide what is the true distribution.) If $F_i(x)$ is the actual distribution, x the observed value and the jth decision is adopted, then the statistician sustains a loss $w_{ij}(x)$, where $w_{ij}(x)$ is a finite real non-negative measurable function of x. If the statistician, on observing the value x, adopts the various decisions with probabilities $\eta_j(x)$ (these being non-negative measurable functions satisfying (1)), then the risk, or expected loss, when $F_i(x)$ is the true distribution function is

$$r_i(\eta) = \sum_{j=1}^n \int_x w_{ij}(x) \eta_j(x) dF_i(x).$$

We again say that the decision function $\eta(x)$ is non-randomized if for every x all but one of the $\eta_j(x)$ vanish.

There is no difficulty in deducing from Theorem 1 the following result.

THEOREM 5. *If the distribution functions are non-atomic[6] then given any decision function* $\eta(x)$, *there exists a non-randomized decision function* $\eta^*(x)$ *equivalent to it, i.e., such that* $r_i(\eta^*) = r_i(\eta)$ *for* $i = 1, 2, \ldots, m$. *If, more-*

over, the loss functions $w_{ij}(x)$ reduce to constants w_{ij}, there exists a non-randomized $\eta^*(x)$ equivalent to $\eta(x)$ simultaneously for all choices of the constants w_{ij}.

6. In a sequential procedure the sample size is not given in advance. In this case the problem may be presented as follows: An infinite sequence of real chance variables $(x_1, x_2, \ldots, x_t, \ldots)$ is distributed according to an unknown one of a finite number of distribution functions $F_i(x_1, x_2, \ldots, x_t, \ldots)$ $(i = 1, 2, \ldots, m)$. The statistician has again a choice of a finite number n of (terminal) decisions. His decision rule δ consists of real, non-negative, measurable functions $\delta_{\nu t}(x_1, x_2, \ldots, x_t)$ $(\nu = 0, 1, \ldots, n;$ $t = 1, 2, \ldots)$ satisfying

$$\sum_{\nu=0}^{n} \delta_{\nu t}(x_1, x_2, \ldots, x_t) = 1 \text{ for all } -\infty < x_1, \ldots, x_t < \infty.$$

The decision rule expressed by δ is interpreted thus: According to the observed value[7] x_1, the statistician decides either to continue experimentation and make another observation, or to stop further experimentation and adopt a terminal decision $j(j = 1, \ldots, n)$ with the respective probabilities $\delta_{01}(x_1)$ or $\delta_{j1}(x_1)$ $(j = 1, \ldots, n)$. If it is decided to continue experimentation, a value of x_2 is observed and it is again decided either to make a further observation or adopt a terminal decision j with respective probabilities $\delta_{02}(x_1, x_2)$ and $\delta_{j2}(x_1, x_2)$, etc., etc. The risk or expected loss if F_i is the true distribution and the decision rule δ is adopted is given by

$$r_i(\delta) = \sum_{t=1}^{\infty} \sum_{\nu=0}^{n} \int_{Y_t} v_{i\nu t}(x_1, \ldots, x_t) \delta_{01}(x_1) \delta_{02}(x_1, x_2) \ldots \delta_{0(t-1)}(x_1 \ldots, x_{t-1}).$$

$$\delta_{\nu t}(x_1, \ldots, x_t) dF_{it}(x_1, \ldots, x_t),$$

where Y_t is the t dimensional space of $x_1, \ldots, x_t$, $v_{i\nu t}(x_1, \ldots, x_t)$ are real finite non-negative measurable functions (representing total loss and cost of experimentation) and $F_{it}(x_1, \ldots, x_t)$ is the joint distribution function of $x_1, \ldots, x_t$ when F_i is the true distribution. The sequential decision rule δ is called non-randomized if for all $x_1, \ldots, x_t$ $(t = 1, 2, \ldots)$ all but one of the $n + 1$ function $\delta_{01}(x_1, \ldots, x_t), \ldots, \delta_{nt}(x_1, \ldots, x_t)$ are equal to zero. Here we have the result:

THEOREM 6. *If the m one-dimensional distribution functions $F_i(x_1)$ $(i = 1, \ldots, m)$ are continuous, then given any sequential decision rule, there exists a non-randomized sequential decision rule equivalent to it. If, moreover, the functions $v_{i\nu t}(x_1, \ldots, x_t)$ reduce to constants $v_{i\nu t}$, there exists a non-randomized decision function, equivalent to the given one, simultaneously for all choices of the constants $v_{i\nu t}$.*

In statistical applications $v_{i0t}(x_1, \ldots, x_t)$ is usually zero, and one considers only decision rules for which the probability is one of terminating

experimentation in a finite number of steps.   Theorem 6 remains valid
if these restrictions are explicitly made.

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[1] I.e., if for some $S \in \mathcal{S}$ and $1 \leq k \leq p$, $\mu_k(S) \neq 0$, then there exists an $S' \subset S$ also belonging to $\mathcal{S}$ and such that $\mu_k(S')$ is neither zero nor equal to $\mu_k(S)$.

[2] *Bull. Acad. Sci. U.R.S.S.*, **4** 465–478 (1940). Cf. also Halmos, P. R., *Bull. Am. Math. Soc.*, **54** 416–421 (1948). The essential part of Liapounoff's theorem was again proved independently by Neyman, J., *C.R. Acad. Sci. Paris*, **222** 843–845 (1946).

[3] Cf., e.g., Neumann, J. v., and Morgenstern, O., *The Theory of Games and Economic Behavior*, 2nd ed., Princeton, 1947.

[4] These Proceedings, **35** 600–605 (1949).

[5] Cf., e.g., Wald, A., *Ann. Math. Stat.*, **20** 165–205 (1949).

[6] This means, in the present case, that no single point is assigned positive probability.

[7] We exclude those procedures in which there is a positive probability of reaching a final decision before making even a single observation.

ON MULTIPLICATIVE PROPERTIES OF A GENERALIZED JACOBI-CAUCHY CYCLOTOMIC SUM

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In this article we shall consider the exponential sum,[1] for $s > 1$,

$$\psi(\alpha_1{}^{\mu_1}, \ldots, \alpha_s{}^{\mu_s}) = \sum_{a_1, \cdots, a_{s-1}} \alpha_s{}^{\mu_s \text{ ind } A} \prod_{i=1}^{s-1} \alpha_i{}^{\mu_i \text{ ind } a_i}; \qquad (1)$$

$$\psi(\alpha_i{}^{\mu_i}) = 1, \qquad (1a)$$

where $\alpha_i = e^{2i\pi/m_i}$; $i = 1, 2, \ldots, s$; $p^n - 1 \equiv 0 \pmod{m_i}$, p is an odd prime; μ_i, $i = 1, 2, \ldots, s$ integers ≥ 0; ind a_i is defined by $g^{\text{ind } a_i} = a_i$, in a finite field of order p^n, designated by $F(p^n)$, or K; g denotes a generator of the cyclic group formed by the non-zero elements of $F(p^n)$ under multiplication, $\alpha_i{}^{\mu_i \text{ ind } (0)} = 0$ for any μ_i, and each a_i ranges independently over each element of K, with $A = 1 - \sum_{i=1}^{s-1} a_i$.

In the article just cited (relation (17)) a relation was found for the number of solutions in $\gamma_1, \gamma_2, \ldots, \gamma_s$ of

$$1 + \sum_{i=1}^{s} g^{j_i + m_i \gamma_i} = 0 \qquad (2)$$

where γ_i is in the range $0, 1, \ldots, (m_i' - 1)$, with $m_i m_i' = p^s - 1$. This result may be regarded as an additive property of the number ψ. Here we shall be more interested in multiplicative properties of it, particularly its decomposition into factors in various ways. The sum ψ is an algebraic integer in the field $k(\alpha)$ with $\alpha = e^{2i\pi/m}$ and $m = p^s - 1$, and from an arithmetic standpoint questions of factorization are often fundamental. The arguments we shall use are, in the main, extensions of known methods for deriving properties of (1) when $s = 2$. In spite of the fact that our ψ seems to be quite a complicated number, relations (15), (17) and (20) turned out to be much simpler than what we originally expected them to be.

We also shall consider

$$\tau(\alpha^\mu) = \sum_{a \in K} \alpha^{\mu \, \text{ind} \, a} \zeta^{\text{tr}(a)}, \tag{3}$$

with μ any integer and we define tr(a) as follows: It is known that $a + a^{(1)} + \ldots + a^{(n-1)} = c$ where c is an element of $F(p)$, and $(k) = p^k$. If we take the residue classes modulo p which form a field isomorphic to $F(p)$ and c maps on a residue class defined by the integer d, then we set $\zeta^{\text{tr}(a)} = \zeta^d$. From the definitions of tr (a) and ind a it follows easily that

ind a + ind b = ind (ab) (mod $(p^s - 1)$), tr (a) + tr (b) = tr($a + b$).

The following relations are known:

$$\sum_{\substack{a \in K \\ a \neq 0}} \zeta^{\text{tr}(ac)} = \begin{cases} -1 \text{ if } c \neq 0, \\ p^s - 1 \text{ if } c = 0; \end{cases} \tag{4}$$

$$\sum_{d=0}^{vm_i-1} \alpha_i^{cd} = \begin{cases} 0; & c \not\equiv 0 \pmod{m_i}, \\ vm_i; & c \equiv 0 \pmod{m_i}; \end{cases} \tag{5}$$

as well as an important property of (3), namely

$$\tau(\alpha_i^\mu)\tau(\alpha_i^{-\mu}) = \alpha_i^{\mu \, \text{ind}(-1)} p^s, \tag{6}$$

where $\alpha_i^\mu \neq 1$. This relation will be generalized in (9).

In order to prove two theorems concerning the factoring of $\psi(\alpha_1^\mu, \alpha_2^\mu, \ldots, \alpha_s^\mu)$ we shall first consider

$$\prod_{i=1}^{s} \tau(\alpha_i^\mu) = \sum_{a_1, \ldots, a_s} \prod_{i=1}^{s} \alpha_i^{\mu \, \text{ind} \, a_i} \zeta^{\text{tr}(a_1 + \cdots + a_s)} \tag{7}$$

Under the assumption that $\prod_{i=1}^{s} \alpha_i^\mu = 1$, this may be reduced by letting $a_i = -b_i a_1, i \geq 2$; (7) becomes,

$$\sum_{a_1, b_1, \ldots, b_s} \left(\prod_{i=1}^{s} \alpha_i^\mu \right)^{\text{ind} \, a_1} \prod_{i=2}^{s} \alpha_i^{\mu \, \text{ind} \, b_i} \cdot \left(\prod_{i=2}^{s} \alpha_i^\mu \right)^{\text{ind} \, (-1)} \zeta^{\text{tr} \, a_1(1-b_2-\cdots-b_s)}.$$

Since $\prod\limits_{i=1}^{s} \alpha_i^{\mu_i} = 1$ and $\alpha_1^{-\mu_1} = \prod\limits_{i=2}^{s} \alpha_i^{\mu_i}$ the above may be written

$$\sum_{b_2, \cdots, b_s} \prod_{i=1}^{s} \alpha_i^{\mu_i \text{ ind } b_i} \alpha_1^{-\mu_1 \text{ ind } (-1)} \cdot \sum_{a_1} \zeta^{\text{tr } a_1(1 - b_2 - \cdots - b_s)}.$$

Using (4) then (7) reduces, when we carry out the second summation, to

$$-\sum \prod_{i=2}^{s} \alpha_i^{\mu_i \text{ ind } b_i} \alpha_i^{-\mu_i \text{ ind } (-1)} + (p^n - 1) \sum' \prod_{i=2}^{s} \alpha_i^{\mu_i \text{ ind } b_i}$$
$$\alpha_1^{-\mu_1 \text{ ind } (-1)}, \quad (8)$$

where in the first summation each b ranges over all elements of $F(p^n)$ such that $1 - b_2 - \ldots - b_s \neq 0$ and in the second, over all elements of $F(p^n)$ where such is not the case.

For $s = 2$, the expression (8) reduces to $p^n \alpha_1^{-\mu_1 \text{ ind } (-1)}$.

For $s > 2$, recombining the terms in (8) gives, for the right-hand member of (7),

$$p^n \sum_{b_2, \cdots, b_{s-1}} \prod_{i=2}^{s-1} \alpha_i^{\mu_i \text{ ind } b_i} \alpha_s^{\mu_s \text{ ind } (1-b_2 \cdots b_s - 1)} \cdot \alpha_1^{-\mu_1 \text{ ind } (-1)} -$$
$$\sum_{b_2, \cdots, b_s} \prod_{i=2}^{s} \alpha_i^{\mu_i \text{ ind } b_i} \alpha_1^{-\mu_1 \text{ ind } (-1)}.$$

The second term is zero unless $\mu_i \equiv 0 \ (m_i)$ for all $i;$ therefore, we may now write,

$$\prod_{i=1}^{s} \tau(\alpha_i^{\mu_i}) = p^n \psi(\alpha_1^{\mu_1}, \ldots, \alpha_s^{\mu_s}) \alpha_1^{\mu_1 \text{ ind } (-1)}$$

$$- \begin{cases} 0, & \text{if } \mu_i \not\equiv 0(m_i) \text{ for some } i > 1, \\ (p^n - 1)^{s-1}, & \text{if } \mu_i \equiv 0(m_i) \text{ for each } i; \end{cases} \quad (9)$$

where $\prod\limits_{i=1}^{s} \alpha_i^{\mu_i} = 1$. For $s = 2$ we obtain (6), using (1a). If we employ the relation,

$$\psi(\alpha_1^{\mu_1}, \ldots, \alpha_s^{\mu_s}) = -\psi(\alpha_2^{\mu_2}, \ldots, \alpha_s^{\mu_s}) \alpha_1^{-\mu_1 \text{ ind } (-1)}$$

where $\prod\limits_{i=1}^{s} \alpha_i^{\mu_i} = 1$, and $\mu_i \not\equiv 0 \ (m_i)$ for any i (the proof of this result will appear in a paper to be published later by one of the authors), we then have[3] by (9),

LEMMA I: *If* $\prod\limits_{i=1}^{s} \alpha_i^{\mu_i} = 1$ *and* $\mu_i \not\equiv 0 \ (m_i)$ *for any* i *then*

$$\psi(\alpha_1^{\mu_1}, \ldots, \alpha_s^{\mu_s}) = \frac{\prod\limits_{i=1}^{s} \tau(\alpha_i^{\mu_i})}{-p^n}. \quad (10)$$

Now we consider (7) under the assumption that $\prod_{i=1}^{s} \alpha_i^{\mu_i} \neq 1$. Let $a_1 + \ldots + a_s = c$. Consider first the case where $c = 0$; if we let $a_i = b_i a$, where $i \geq 2$ then since $1 + b_2 + \ldots + b_s = 0$ we may write (7), on the right, under the above assumption,

$$\sum_{a_1, b_1, \cdots, b_{s-1}} \left(\prod_{i=1}^{s} \alpha_i^{\mu_i} \right)^{\text{ind } a_1} {}^{s-1}_{\prod_{i=2}} \alpha_i^{\mu_i \text{ ind } b_i} \cdot \alpha_s^{\mu_s \text{ ind } (-1 - b_1 - \cdots - b_{s-1})}.$$

Since $\prod_{i=1}^{s} \alpha_i^{\mu_i} \neq 1$, $\sum_{a_1} \left(\prod_{i=1}^{s} \alpha_i^{\mu_i} \right)^{\text{ind } a_1} = 0$ thus in the case where $c = 0$, (7) equals zero. Consider now the case where $c \neq 0$. If we let $a_i = c b_i$, then since $b_1 + b_2 + \ldots + b_s = 1$, we may under these conditions write the right-hand member of (7) as

$$\sum_{b_1, \cdots, b_{s-1}} \prod_{i=1}^{s-1} \alpha_i^{\mu_i \text{ ind } b_i} \alpha_s^{\mu_s \text{ ind } (1 - b_1 - \cdots - b_{s-1})} \cdot \sum_{c} \left(\prod_{i=1}^{s} \alpha_i^{\mu_i} \right)^{\text{ind } c} \zeta^{\text{tr } c}.$$

which in view of (1) and (3) may be written,

$$\psi(\alpha_1^{\mu_1}, \ldots, \alpha_s^{\mu_s}) \cdot \tau \left(\prod_{i=1}^{s} \alpha_i^{\mu_i} \right).$$

Thus we now have

LEMMA II: *If* $\prod_{i=1}^{s} \alpha_i^{\mu_i} \neq 1$, *then*

$$\psi(\alpha_1^{\mu_1}, \ldots, \alpha_s^{\mu_s}) = \frac{\prod_{i=1}^{s} \tau(\alpha_i^{\mu_i})}{\tau \left(\prod_{i=1}^{s} \alpha_i^{\mu_i} \right)}. \tag{11}$$

From this we immediately obtain[3]

$$\psi \bar{\psi} = p^{\pi(s-1)} \tag{12a}$$

provided $\alpha_i^{\mu_i} \neq 1, i = 1, \ldots s$; $\prod_{i=1}^{s} \alpha_i^{\mu_i} \neq 1$; on using (6).

Set, if $s > t$,

$$\psi_{t, s} = \psi \left(\prod_{i=1}^{t} \alpha_i^{\mu_i}, \alpha_{t+1}^{\mu_{t+1}}, \ldots, \alpha_s^{\mu_s} \right). \tag{12}$$

We now proceed to factorize ψ, distinguishing four cases, I–IV. Throughout the discussion of the four cases we assume $s > t > 1$ and $\mu_i \neq 0 \pmod{m_1}$ for each i.

I. If $\prod_{i=1}^{s} \alpha_i^{\mu_i} = 1$ then by (10), and using the notation indicated in (12), we have

$$\psi_{1,\,s} \; = \; \frac{\displaystyle\prod_{i\,=\,1}^{t} \tau(\alpha_i{}^{\mu_i}) \cdot \prod_{i\,=\,t+1}^{s} \tau(\alpha_i{}^{\mu_i})}{-p^{\pi}} \tag{13}$$

Suppose $\displaystyle\prod_{i\,=\,1}^{t} \alpha_i{}^{\mu_i} = 1$. If $t = s - 1$, then $\mu_s \equiv 0 \ (m_s)$, which is contrary to the original assumption; thus we may assume $t < s - 1$ noting that $s > 2$ by hypothesis. The right hand member of (13) then becomes by (10), noting that $\alpha_{t+1}^{\mu_{t+1}} \ldots \alpha_s^{\mu_s} = 1$,

$$\psi_{1,\,s} \; = \; -p^{\pi}\psi_{1,\,t} \cdot \psi(\alpha_{t+1}^{\mu_{t+1}}, \, \ldots, \, \alpha_s^{\mu_s}). \tag{13a}$$

II. Suppose $\displaystyle\prod_{i\,=\,1}^{t} \alpha_i{}^{\mu_i} \neq 1$, with $\displaystyle\prod_{i\,=\,1}^{s} \alpha_i{}^{\mu_i} = 1$. If $t < s$, (13) may be written as

$$\frac{\displaystyle\prod_{i\,=\,1}^{t} \tau(\alpha_i{}^{\mu_i})}{\tau\left(\displaystyle\prod_{i\,=\,1}^{t} \alpha_i{}^{\mu_i}\right)} \cdot \frac{\tau\left(\displaystyle\prod_{i\,=\,1}^{t} \alpha_i{}^{\mu_i}\right) \displaystyle\prod_{i\,=\,t+1}^{s} \tau(\alpha_i{}^{\mu_i})}{-p^{\pi}}\;,$$

which becomes by (10) and (11)

$$\psi_{1,\,t}\psi_{t,\,s} \; = \; \psi_{1,\,s}. \tag{13b}$$

III. If $\displaystyle\prod_{i\,=\,1}^{s} \alpha_i{}^{\mu_i} \neq 1$ with $\displaystyle\prod_{i\,=\,1}^{t} \alpha_i{}^{\mu_i} = 1$ then by (10) we have

$$\psi_{1,\,s} \; = \; \frac{\displaystyle\prod_{i\,=\,1}^{t} \tau(\alpha_i{}^{\mu_i}) \prod_{i\,=\,t+1}^{s} \tau(\alpha_i{}^{\mu_i})}{\tau\left(\displaystyle\prod_{i\,=\,1}^{s} \alpha_i{}^{\mu_i}\right)}\;. \tag{14}$$

Since $\tau\left(\displaystyle\prod_{i\,=\,1}^{s} \alpha_i{}^{\mu_i}\right) = \tau\left(\displaystyle\prod_{i\,=\,t+1}^{s} \alpha_i{}^{\mu_i}\right)$, (14) may be written as

$$-p^{\pi}\frac{\displaystyle\prod_{i\,=\,1}^{t} \tau(\alpha_i{}^{\mu_i})}{-p^{\pi}} \; \frac{\displaystyle\prod_{i\,=\,t+1}^{s} \tau(\alpha_i{}^{\mu_i})}{\tau\left(\displaystyle\prod_{i\,=\,t+1}^{s} \alpha_i{}^{\mu_i}\right)}\;,$$

which becomes by (10) and (11)

$$\psi_{1,\,s} \; = \; -p^{\pi}\psi_{1,\,t} \, \psi(\alpha_{t+1}^{\mu_{t+1}}, \, \ldots, \, \alpha_s^{\mu_s}). \tag{14a}$$

IV. Suppose $\displaystyle\prod_{i\,=\,1}^{t} \alpha_i{}^{\mu_i} \neq 1$ with $\displaystyle\prod_{i\,=\,1}^{s} \alpha_i{}^{\mu_i} \neq 1$; (14) may be written

$$\frac{\prod\limits_{i=1}^{t} \tau(\alpha_i^{\mu_i})}{\tau\left(\prod\limits_{i=1}^{t} \alpha_i^{\mu_i}\right)} \frac{\tau\left(\prod\limits_{i=1}^{t} \alpha_i^{\mu_i}\right) \prod\limits_{i=t+1}^{s} \tau(\alpha_i^{\mu_i})}{\tau\left(\prod\limits_{i=1}^{s} \alpha_i^{\mu_i}\right)} ,$$

which becomes by (11)

$$\psi_{1,t}\psi_{t,s} = \psi_{1,s}. \tag{14b}$$

Thus we have, since results (13a), (13b) and (14a), (14b) are the same, respectively:

THEOREM I: *If $\mu_i \not\equiv 0(m_i)$ and $s > t > 1$, then*

$$\psi_{1,s} = \begin{cases} \psi_{1,t}\psi_{t,s}, \text{ if } \prod\limits_{i=1}^{t} \alpha_i^{\mu_i} \neq 1; \\ -p^n \psi_{1,t}\psi \ (\alpha_{t+1}^{\mu_{t+1}}, \ldots, \alpha_s^{\mu_s}) \text{ if } \prod\limits_{i=1}^{t} \alpha_i^{\mu_i} = 1, \end{cases} \tag{15}$$

the symbols used being defined in (1) and (12).

Suppose $t_1 > t_2 > \ldots > t_k, s > t_i \geq 2$, are the distinct values of t for which $\prod\limits_{i=1}^{t} \alpha_i^{\mu_i} = 1$ and assume also that $\mu_i \not\equiv 0(m_i)$. By Theorem I we may write

$$\psi_{1,s} = \psi_{1,t+1} \psi_{t+1,s}. \tag{16}$$

By continually reapplying said Theorem, (16) becomes, since t_1 exceeds any other t,

$$\psi_{1,s} = -p^n \psi_{1,t_1} \psi_{t_1+1,t_1+2} \ldots \psi_{s-1,s}.$$

Repeated application of this relation and (15) gives

$$\psi_{1,s} = (-p^n)^k \frac{\psi_{1,2} \psi_{2,3} \ldots \psi_{s-1,s}}{\psi_{t_1,t_1+1} \ldots \psi_{t_k,t_k+1}},$$

but $\psi_{t_1,t_1+1} = \sum\limits_a (1)^{\text{ind } a} (\alpha_{t+1}^{\mu_{t+1}})^{\text{ind } (1-a)}$, and since $\alpha_{t_1+1}^{\mu_{t_1+1}} \neq 1, \psi_{t_1,t_1+1} = -1$, then we have[4]

THEOREM II: *If $\mu_i \not\equiv 0(m_i), i = 1, 2, \ldots, s, and k represents the number of distinct t's for which $\prod\limits_{i=1}^{t} \alpha_i^{\mu_i} = 1$ where $t < s$, then*

$$\psi_{1,s} = p^{kn} \psi_{1,2} \psi_{2,3} \ldots \psi_{s-1,s}, \tag{17}$$

the symbols used being defined in (1) and (12).

The condition, $\mu_i \not\equiv 0(m_i)$, does not constitute a restriction on the generality of this result since we can reduce a ψ number for which $\mu_i \equiv 0(m_i)$ for some i's to a ψ number involving only those $\alpha_i^{\mu_i}$ for which $\mu_i \not\equiv 0(m_i)$.

For the case where $m_1, m_2, \ldots, m_t$ are prime each to each then $\prod\limits_{i=1}^{t} \alpha_i^{\mu_i} = 1$ if and only if $\mu_i \equiv 0(m_i)$, $i = 1, 2, \ldots, t$. Hence we have the

COROLLARY, *If $m_1, m_2, \ldots, m_s$, defined in connection with (1), are prime each to each, then*

$$\psi_{1,\,s} = \psi_{1,\,2}\,\psi_{2,\,3}\ldots\psi_{s-1,\,s} \tag{18}$$

From Theorem II we obtain in general a great variety of decompositions of ψ by renumbering the quantities

$$\alpha_1^{\mu_1}, \alpha_2^{\mu_2}, \ldots, \alpha_s^{\mu_s},$$

as, say,

$$\alpha_{h_1}^{\mu h_1}, \alpha_{h_2}^{\mu h_2}, \ldots, \alpha_{h_s}^{\mu h_s},$$

the latter being any permutation of the original set, and letting $\alpha_i^{\mu_i}$ be replaced by $\alpha_{h_i}^{\mu h_i}$ in the proof of (17); $i = 1, 2, \ldots, s$.

The form of (17) enables us to obtain a number of multiplicative results concerning $\psi(\alpha_1^{\mu_1}, \ldots, \alpha_s^{\mu_s})$ from the multiplicative properties of $\psi(\alpha_h^{\mu h}, \alpha_k^{\mu k})$. For example let m be the least common multiple of $m_1, m_2, \ldots, m_s$, then any $\psi_{h,\,h+1}$ may be written in the form

$$\psi_{h,\,h+1} = \sum_{a\,\epsilon\,K} \beta^{b \text{ ind } a + d \text{ ind } (1 - a)} \tag{19}$$

where $\beta = e^{2i\pi/m}$ and b and d are some integers. Suppose we define a number $\psi_{h,\,h+1}^{(r)}$ by using $F(p^{rn})$ in place of $F(p^n)$ in (19), then further assume that $g_r^{c_r} = g^c$ where g_r is a multiplicative generator of the cyclic group formed by the non-zero elements of $F(p^{rn})$, g has the same property for $F(p^n)$, and also

$$c_r = \frac{p^{rn} - 1}{m}, \qquad c = \frac{p^n - 1}{m}.$$

Then H. H. Mitchell[3] showed that

$$\psi_{h,\,h+1}^{(r)} = (-1)^{r-1}(\psi_{h,\,h+1})^r$$

unless both b and d are $\equiv 0(\text{mod } m)$. Using this together with (17) gives immediately

$$(\psi_{1,\,s})^r = (-1)^{(s-1)(r-1)}p^{(r-1)kn}\psi_{1,\,s}^{(r)} \tag{20}$$

for $\mu_i \not\equiv 0 \ (\text{mod } m_i)$, $i = 1, 2, \ldots, s$, and k is defined as in Theorem II.

[1] Vandiver, these PROCEEDINGS, 36, 144 (1950), with references there given.

[2] Weil, *Bull. Am. Math. Soc.*, 55, 501 (1949), last relation on said page, and relation (13) of the reference given in our first footnote. Our relation (9) of the present paper, not given by Weil, is more convenient than (10) for some purposes.

[3] Weil, *loc. cit.*, p. 501, gave an argument different at least in form to derive a corresponding property to this for his number $j(\alpha)$ which is a special case of our number ψ of (1).

[4] For $n = 1$, the nearest approach to the process used here seems to be found in H. J. S. Smith's Report on the Theory of Numbers, Collected Works, Vol. I, 271–272, Oxford (1894); reproduced by P. Bachmann, *Die Lehre von der Kreisteilung*, 279–281, Leipzig and Berlin (1921). However, our number ψ of (1) for $n = 1$ is not exhibited explicitly by them in terms of the α's alone, but is defined by means of the $\tau(\alpha^x)$ of our relation (3).

[5] *Ann. Math.*, 18, 120 (1917).

THE SOLUTION OF A CERTAIN GENERAL TYPE OF INTEGRAL EQUATION

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The following integral equation arose in an engineering design problem:

$$\int_0^1 [f''(1)f(u) + 2f'^2(u)]du = 0. \tag{1}$$

The only restriction placed on the function $f(u)$ is that it be analytic.

If we assume that $f(u)$ has the form

$$f(u) = F(u) + Au, \tag{2}$$

where $F(u)$ is any given analytic function, then equation (1) takes the form

$$\int_0^1 [F''(1)F(u) + AuF''(1) + 2F'^2(u) + 2A^2 + 4AF'(u)]du = 0 \tag{3}$$

or, on performing the integrations

$$2A^2 + 4A[F(1) - F(0) + \tfrac{1}{2}F''(1)] + \int_0^1 [F''(1)F(u) + 2F'^2(u)]du = 0. \tag{4}$$

This quadratic equation in A has two solutions for every given $F(u)$.

We have thus found the expression for A which makes equation (2) become a solution of equation (1).

It is obvious that a solution of Type (2) exists for a much more general type of integral equation than (1). Consider a function ϕ which is rational and analytic in $f(u)$ and its various derivatives but does not involve the independent variable u explicitly. The integral equation

$$\int_0^1 \phi[f(u), f'(u), f''(u), \ldots]du = 0 \tag{5}$$

will have a solution of Type (2), since by substituting equation (2) and its derivatives for $f(u)$ and its derivatives and performing the integrations, we obtain an algebraic equation for A which can be solved at least in principle.

It should be possible to extend these results to still more general types of integral equations.

EXTRACTION OF A MATING REACTION INHIBITING AGENT FROM PARAMECIUM CALKINSI*

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Since interaction of mating-type substances at the surfaces of conjugating paramecia may initiate the physiological changes of fertilization in these animals,[1] a detailed study of the nature and manner of interaction of these substances is warranted. So far all attempts to obtain an active extract of mating substance from paramecia have failed.[2, 3] Furthermore all mating reactivity disappears when these animals are thoroughly broken up. This apparent disappearance of mating substance activity from both extract and residue suggests the release of some mating substance inhibiting agent. Such an agent has now been obtained from homogenized *Paramecium calkinsi*. The presence of this agent can account for disappearance of mating substance activity in certain, but not in all, extraction procedures.

Preparation of the Agent.—*Paramecium calkinsi* were grown in a 2/5 sea water-baked lettuce infusion which had previously been inoculated with *Aerobacter aerogenes*. To prepare paramecium extracts the animals in four to six liters of paramecium culture were concentrated to 25–50 ml. by passing the culture through a porous-grade Berkefeld filter (the senior author's method has been described fully by Sonneborn).[4] The animals were further concentrated by centrifugation, washed in 2/5 sea water and taken up in one to three ml. of 2/5 sea water. These concentrated animals were then homogenized or were lyophilized[5] and later taken up in distilled water and homogenized. The crude homogenate was then centrifuged or passed through a sintered glass filter to give an opalescent supernatant or filtrate.

The most practical method for detecting any mating-type substance inactivating agent is to treat reactive dead paramecia with the agent, wash the dead animals free of the agent and then test them for specific mating reactivity with living animals. When testing paramecium homogenates for mating substance inactivating action the use of reactive dead animals in preference to living animals is essential because living animals may be killed by a non-mating-type specific heat labile auto-toxin frequently present in homogenates (Metz, unpublished). This introduces a complicating factor which cannot be controlled. If living animals are not killed by the homogenate, they can feed upon it with consequent loss of mating reactivity due to overfeeding.

The dead reactive paramecia used in these experiments were prepared

by treating cultures of *P. calkinsi* with formalin or picric acid (19 or 15 volumes of culture to one volume of formalin or saturated picric acid solution, respectively) for one hour.   After the treatment the animals were washed in 2/5 sea water and suspended in this saline.   Properly selected cultures of reactive Type I *P. calkinsi* that have been killed in this manner give very strong mating reactions with living Type II animals.   These dead animals will even induce meiosis, macronuclear breakdown and pseudoselfing pair formation in living Type II animals.[3]   Formalin-killed Type II animals at best give only moderately strong reactions with living Type I animals, whereas picric acid-killed Type II animals can give very strong reactions.

To test for inhibiting action one to several volumes of the opalescent extracts were mixed with a given volume of strongly reactive dead paramecia (usually two drops containing roughly 1000 dead animals). After an exposure of one to two hours the extract was withdrawn from the treated dead animals.   These were then washed in 2/5 sea water and tested for mating reactivity by mixing with living reactive animals of the same and opposite mating type.   Generally such extract-treated dead animals failed to give mating reactions or at best gave only weak transitory reactions as compared to control dead animals buffered at the pH (5.5) of the extract.

Relation of the Inhibiting Agent to the Mating Substances.—Apparently the mating substance inhibiting agent of the extracts is not the mating substance of the extracted animals.   This follows from the fact that the inhibiting agent is non-specific.   Thus the extract prepared from mating Type I *P. calkinsi* inhibited dead reactive animals of both Type I and Type II.   Moreover *P. calkinsi* I extract inhibited formalin-killed reactive variety 4 (Types VII and VIII) *Paramecium aurelia*.   Extracts prepared from Type II *P. calkinsi* were also non-specific in action.   If the inhibiting agent were the mating substance of the extracted animals, it should show mating type specificity.   It would be expected to react with and inhibit only animals of the complementary mating type, not animals of the mating type from which the extract was prepared.

In view of the non-specific character of the inhibiting effect, one system, namely the action of Type I extract on Type I animals, was selected for further study.

Mode of Action of the Agent.—The inhibiting action of homogenates was not altered by repeated centrifugation or by passage through a sintered glass filter.   Thus the inhibiting action cannot be attributed to a simple mechanical factor such as clogging of the cilia of the treated dead animals with debris.   Therefore it is concluded that inhibition results from chemical combination of the mating substance with some agent in the extracts.

Apparently the non-specific mating substance inhibiting agent does not

act by combining with the mating substance of dead animals in antigen-antibody-like fashion. This follows from the fact that all attempts to absorb the inhibiting agent of Type I extracts by living or dead Type I paramecia have failed, and suggests that the agent may inactivate the mating substance by enzymatic action. Furthermore the agent acts slowly and only in low dilution. This is indicated by the experiment presented in table 1. The extract used in this experiment was prepared by concentrating six liters of culture (0.8 cc. packed paramecia) and homogenizing the animals in 3 cc. of 2/5 sea water after lyophilization.

Unfortunately there is no convenient objective assay method available for determining the amount of active mating substance present on dead or living paramecia. However, the relative degree of reactivity as indicated subjectively in table 1 shows that little if any inactivation occurred during the first 20 minutes of exposure to extract even in the highest extract concentration. Sixty minutes' exposure to the extract were required for

TABLE 1

EFFECT OF EXTRACT CONCENTRATION ON RATE OF INHIBITION OF FORMALIN-KILLED PARAMECIUM CALKINSI TYPE I BY P. CALKINSI TYPE I EXTRACT

TIME:	20 MIN.	40 MIN.	60 MIN.
Extract dilution			
5/7	++++	+	±
2.5/7	++++	+++	++
1.25/7	++++	++++	+++
0.65/7	++++	++++	++++
pH 5.5 buffer	++++	++++	++++

nearly complete inactivation of the formalin-killed Type I animals. The reactivity of the test animals in the fourfold (0.65/7) extract dilution was not significantly different from that of controls in buffer solution even after a 60-minute treatment with the extract.

It is apparent from this experiment that the inhibiting agent acts slowly even at the lowest dilutions used and that the rate of inactivation is a function of the concentration of the agent.

Properties of the Agent. — Preliminary attempts to characterize the inhibiting agent of Type I animals show that it is heat labile to the extent that its action is rapidly destroyed at 100°C. It is non-dialyzable and evidently stable in absolute acetone since paramecia which have been lyophilized, treated with absolute acetone, dried and finally extracted yield active preparations of the inhibiting agent. Extracts of formalin-killed Type I animals, however, do not inhibit Type I or Type II animals. Attempts to salt out the agent with ammonium sulfate have failed. The $(NH_4)_2SO_4$ precipitates and supernatants were inactive both separately and when combined. Probably the agent was inactivated in the process of removing the salt.

These fragmentary data indicate that the inhibiting agent is a rather labile substance of fairly high molecular weight and suggest that it may be a protein. This is in accord with the view that its action is enzymatic.

Discussion.—As mentioned previously, the mating substance inhibiting agent cannot be the mating substance of the extracted animals. This follows from the non-specific character of its action, from the fact that it cannot be extracted from formalin-killed reactive *Paramecium calkinsi* and that it can be obtained from animals which are not in mating condition. The presence of this agent can account for failure to obtain active extracts of mating substance from homogenized living or lyophilized animals. However, it cannot account for disappearance of mating substance activity in both extract and residue of homogenized animals that have received sufficiently harsh treatment (i.e., formalin) to destroy the inhibiting agent without destroying the mating substance. Disappearance of mating substance activity under these conditions cannot be explained readily except by assuming that essential structural relations of the cell surface are destroyed by the mechanical extraction procedure used. At present there is no evidence that any such gross steric factors are essential for mating substance activity.

This study suggests that the mating substance inhibiting agent is enzymatic in action. If the agent should prove to be an enzyme and if the chemical nature of its action could be demonstrated with a well-defined artificial substrate, knowledge of the nature of the mating substance might be considered advanced. Such an enzyme might be concerned specifically with the characteristic appearance and disappearance of mating reactivity in normal living animals in accordance with their nutritional state. It is also possible that the agent is in the nature of a "digestive" enzyme which bears no physiological relation to the mating substance. Either of these possibilities is of sufficient interest to warrant further investigation of the mating substance inactivating agent.

Summary.—Extracts of *Paramecium calkinsi* which inhibit the mating reactivity of formalin or picric acid-killed Paramecium are described. The extracts are not species or mating type specific in action. Therefore it is concluded that the active agent in the extracts is not the mating substance of the extracted animals. Since the inhibiting agent is not absorbed by paramecia it is suggested that it may be an enzyme. The possible relation of the inhibiting agent to the mating substance is discussed.

[1] Metz, C. B., and Foley, M. T., *J. Exptl. Zoöl.* 112, 505 (1949).

[2] Metz, C. B., *Anat. Record*, 94, 347 (1946).

[3] Metz, C. B., *Am. Naturalist*, 82, 85 (1948).

[4] Sonneborn, T. M., *J. Exptl. Zoöl.* (in press) (1950).

[5] Metz, C. B., and Fusco, E. M., *Biol. Bull.*, 97, 245 (1949).

ON THE RATE OF PASSIVE SINKING OF DAPHNIA

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The resistance encountered by a body falling in a viscous liquid is given approximately by dimensional analysis as

$$R = kd^a v^t \rho^{t-1} \mu^{2-t} \qquad (1)$$

where d is an appropriate linear dimension, v the velocity of the body, ρ the density of the medium and μ the viscosity of the medium. When the resistance exactly balances the force of gravity the velocity becomes constant and is given by

$$v^t = Kd^{3-t}(\rho' - \rho)\rho^{1-t}\mu^{t-2}, \qquad (2)$$

where K is constant for bodies of the same shape and ρ' is the density of such bodies.

For small bodies falling slowly in media of high viscosity, the resistance will be determined by the viscosity of the medium but not by the density. In this case $q = 1$ and

$$v \propto d^2(\rho' - \rho)\mu^{-1}. \qquad (3)$$

This is the general form of the relationship deduced just a century ago by Stokes for a small sphere falling slowly.

For large bodies falling so rapidly that viscosity is of no consequence in comparison with the inertia of the liquid, $q = 2$ and

$$v \propto \sqrt{d(\rho' - \rho)\rho^{-1}}. \qquad (4)$$

This is the general form of the relationship deduced by Newton late in the 17th century, and which holds with fair accuracy for some cases of large rapidly falling bodies.[1]

In general Stokes' law corresponds to laminar flow and Newton's law to turbulent flow of the liquid past the body. It is generally conceded that Stokes' law holds with considerable accuracy when Reynolds' Number, N, < 0.5, where

$$N = \frac{dv\rho}{\mu}. \qquad (5)$$

Newton's law, however, does not apply immediately when N exceeds 0.5, and over a certain fairly wide interval there is empirical evidence that $q = \dfrac{3}{2},$[1] and therefore

The passive sinking of D. sp. 1 both in its relation to the linear dimensions of the animal and in its relation to the viscosity of the medium thus clearly obeys the generalized form of Stokes' law.   It is probable that the largest individuals, for which the length is 1.79 mm., and the velocity of sinking 0.24 cm. per sec. when the viscosity is 0.0097 poise, must come very close to the upper limit for the validity of the law.   In such a case Reynold's Number, N, can hardly be very much less than $\dfrac{0.179 \times 1 \times 0.24}{0.0097} =$ 4.43.

In figure 3 the mean sinking speed of three narcotized specimens of D. *pulex*, falling with closed antennae, are plotted from Eyden's data.[4] A fourth specimen which changed density owing to reproduction during the experiment has been excluded.   The open circles represent the mean values, for the midpoint in the size ranges, of two experiments not reported in detail. The whole series of five points falls very close to a straight line of slope 1.   According to Allen's law, the velocity varies as the appropriate linear dimension less a quantity ξd_i as has been indicated above. In the present experiment if the density difference be taken as 0.02,[5] d_i will be given by (8) as 0.028 cm.   For a sphere ξ is 0.4, for an irregular body 0.28;[6] a value for a falling *Daphnia* of 0.3 would seem reasonable, implying a correction of 0.08 mm. to the linear dimensions of the animals employed.   The effect of such a correction would be to reduce the slope of the line of best fit in figure 3 very slightly below unity. It is, however, practically certain that the ratio of the length of the animal to the unknown diameter of the tube employed by Eyden
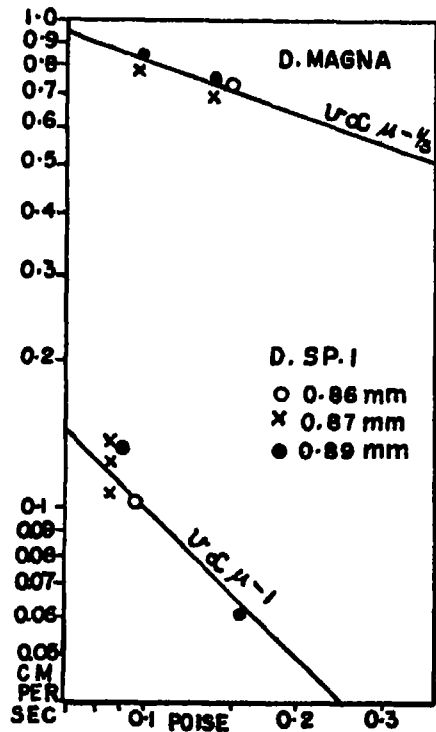

FIGURE 4

Relation of the sinking velocity of *Daphnia magna* (from the data of Bowkiewicz[7]) and of D. sp. 1, to viscosity.   The crosses, open circles and solid circles indicate different individuals.

was at least as great, and probably somewhat greater, in her experiments, than was the maximum ratio in those on D. sp. 1.   The effect of the

Ladenburg correction on Eyden's data would be to increase the slope of the line of best fit. The most reasonable interpretation of figure 3, therefore, is that these two small errors balance each other and that Allen's law holds, at least approximately.

The upper part of figure 4 indicates the relationship between mean sinking velocity and viscosity in experiments by Bowkiewicz with three specimens of *D. magna*, about 2 mm. long. These animals were narcotized, but unlike Eyden's, had open antennae. They certainly were considerably denser than the *D. pulex* employed in her experiments. Bowkiewicz concluded that the sinking speed in this case varied inversely as the square root of the viscosity. He therefore supposed that Newton's law held in this case. Both conclusions are erroneous. Newton's law contains no viscosity term while examination of the figure clearly shows that at least for narcotized animals the sinking speed is inversely proportional to the cube root of the viscosity. Some of Bowkiewicz' experiments with fixed *Daphnia* diverge somewhat from this relationship but so far as living animals are concerned the relationship of the velocity to viscosity is exactly as would be expected from Allen's Law. In these experiments the upper limit of Reynolds' Number, calculated as before, using the length of the animal as the appropriate linear dimension, lies between 10 and 16.

In figure 2 we have given observations on a few specimens of *Daphnia* sp. 2[7] from Bantam Lake, Conn. These animals are somewhat larger than most specimens of *D*. sp. 1 though the increment is mainly due to the taller helmet of *D*. sp. 2. The available evidence seems to suggest some intermediate condition between Stokes' and Allen's laws but it is uncertain whether there is a more or less abrupt transition between the two possible relationships or whether some intermediate value of q, between 1 and 1.5, is implied.

Summary.—The velocity of passive falling of narcotized specimens of small limnoplanktonic *Daphnia* (*D*. sp. 1) is proportional to the square of the linear dimensions and inversely proportional to the viscosity, as implied by Stokes' law; the velocity of passive falling of large pond *Daphnia* (*D. pulex, D. magna*) is nearly proportional to the linear dimensions and inversely proportional to the cube root of the viscosity, as implied by Allen's law.

[1] Allen, H. S., "The Motion of a Sphere in a Viscous Fluid," *Phil. Mag. London, Edinburgh, Dublin*, ser. v, 50, 323–338, 519–534 (1900).

[2] Bowkiewicz, J., "Schwebephase in der Bewegung der Cladoceren und Viskosität des Wassers," *Int. Rev. ges. Hydrobiol. Hydrogr.*, 22, 146–156 (1929).

[3] *Daphnia* sp. 1 would be called *D. longispina* var. *hyalina* Leydig 1860 form *mendotae* according to Birge (in Ward and Whipple's *Fresh Water Biology*), 1918. A redescription of this species is in preparation by one of us (J. L. B.).

[4] Eyden, D., "Specific Gravity as a Factor in the Vertical Distribution of Plankton,"

Proc. Cambridge Phil. Soc. Biol. Sci. (later *Biol. Rev. Cambridge Phil. Soc.*), 1, 49–55 (1923).

⁴ Lowndes, A. G., "The Displacement Method of Weighing Living Aquatic Organisms," *J. Mar. Biol. Assoc. U. K. Plymouth*, 23, 555–574 (1942).

⁶ DallaValle, J. M., *Micromeritics. The Technology of Fine Particles*, Pitman Publishing Co., New York and London, (1948), XXVIII + 555 pp.

⁷ This species has not been described. Woltereck, *Trans. Wisc. Acad. Sci. Madison*, 27, 487–522 (1932) gives an outline drawing of a *Daphnia* undoubtedly referable to this species as figure 26 (Plate XVI). He has named it *D. longispina apicata* form *nasuta* without any description. A description is in preparation by J. L. B.

NATIONAL ACADEMY OF SCIENCES: MINUTES OF THE MEETING FOR ORGANIZATION, APRIL, 1863

BY EDWIN B. WILSON

Communicated March 13, 1950

The Report of the National Academy of Sciences for the Year 1863 published in Washington by the Government Printing Office, 1864, contains only the briefest reference to the organization meeting of the Academy It has seemed to the Home Secretary and the Chairman of the Committee on Revision of Constitution that the members of the Academy who have recently been engaged upon a reorganization of the Constitution and By-laws might be interested in having for reference the Minutes of the Meeting for Organization and a copy of the initial Constitution and Bylaws as considered at that meeting in Committee of the Whole and finally passed at the stated meeting on the 6th of January, 1864. The address of the Hon. Henry Wilson to which reference is made in the Minutes is also appended.

In presenting this material of historic significance to the members of the Academy and to scientists at large, it may be well to call attention to a few remarks of President A. D. Bache in the first annual report of the Academy as revealing the spirit in which the original members and officers approached their tasks.

"The want of an institution by which the scientific strength of the country may be brought, from time to time, to the aid of the government in guiding action by the knowledge of scientific principles and experiments, has long been felt by the patriotic scientific men of the United States. No government of Europe has been willing to dispense with a body, under some name, capable of rendering such aid to the government, and in turn of illustrating the country by scientific discovery and by literary culture.

"It is a remarkable fact in our annals that, just in the midst of difficulties which would have overwhelmed less resolute men, the 37th Congress of the United States, with an elevated policy worthy of the great nation which

they represented, took occasion to bring the scientific men around them in council on scientific matters, by creating the National Academy of Sciences. Such has been the way in which the public mind has been stirred before in the annals of other countries, leading to the organization of government systems of education, science, art, and literature, to be encouraged and perfected when more peaceful and prosperous times recurred.

. . . .

"It will be seen, by the spirit and the words of our laws enacted by the authority by the charter, that members of the National Academy put their time and talents at the disposal of the country in no small or stinted measure, freely, fully, by the binding authority of an oath, asking no compensation therefor but the consciousness of contributing to judicious action by the government on matters of science. The more the wealth of such men can be drawn out from the treasury of their knowledge the richer the nation will be; and I, for one, do not fear that even the suggestions which may be made to Congress, of subjects in which that knowledge may be most profitably employed for our country and times, will be subject to any supposed taint of self-seeking as to power or influence. Subject to the taint of supposed desire for remuneration it cannot be, as our charter and all our laws look away from such a centre."

Minutes of the Proceedings
of
The National Academy of Sciences
at the
Meeting held for Organization
in the
Chapel of the New York University
on the
22d, 23d, & 24th days
of
April 1863

In accordance with an appointment made by the Hon. Henry Wilson of Mass. of which due notice had been given, the members of The National Academy of Sciences met in the Chapel of the New York University at 11 a. m. on Wednesday, April 22nd, 1863.

Mr. Wilson was present and called the meeting to order; and after a brief statement of the origin and history of the Bill incorporating the Academy, which was by him introduced into the Senate of the United States called upon Prof. Agassiz, the first named in the Bill, to take the chair. Prof. Agassiz on account of temporary ill health declined the honor tendered him; and after some few pertinent remarks upon the importance of the establishment of the Academy to the progress of Science, and to the general interests of the country, nominated Prof. Joseph Henry of Washington, as chairman, & Prof. Alexis Caswell of R. I. as secretary, pro tempore, both of whom were appointed and entered upon their respective duties.

The following Resolutions offered by Dr. Gibbs of New York were unanimously adopted:

Resolved, that the Academy accepts the Act of Incorporation, & hereby declares its intention of entering with earnestness & devotion upon the high course marked out for it by Congress.

Resolved, that the thanks of the Academy be presented to the Hon. Henry Wilson for the statesmanlike and successful action in the Senate of the United States for the establishment of a National Academy of Sciences; & that he be invited at this and at all other times when agreeable to him, to be present at, and assured of a cordial welcome at, the meetings of the Academy.

The Bill of Incorporation was then read.   Thirty-two (32) corporate members were present and answered to their names as follows.

Agassiz, L.	Frazer, J. F.	Alexander, S.	Rogers, F.
Bache, A. D.	Gibbs, W.	Barnard, F. A. P.	Rogers, R. E.
Barnard, J. G.	Gilliss, J. M.	Hubbard, J. S.	Rogers, W. B.
Bartlett, W. H. C.	Gould, B. A.	Leidy, J.	Rutherfurd, L. M.
Caswell, A.	Guyot, A.	Lesley, J. P.	Saxton, J.
Coffin, J. H. C.	Hall, J.	Newberry, J. S.	Silliman, B., Jr.
Dana, J. D.	Henry, J.	Newton, H. A.	Strong, T.
Davis, C. H.	Hilgard, J. E.	Peirce, B.	Winlock, J.

On motion of Prof. B. Peirce it was *Voted* that a committee of nine be appointed by the chair to draft & report a plan of organization.   The chair appointed the following: A. Caswell, A. D. Bache, W. B. Rogers, W. Gibbs, J. F. Frazer, B. Silliman, Jr., B. A. Gould, B. Peirce and L. Agassiz.

Opportunity was given for a general interchange of views upon the plan most proper to be adopted.

On motion of Dr. Gould it was *Voted* that members having any propositions to submit to the committee be requested to present them in writing.

On motion of Dr. Gibbs it was *Voted* that the chairman & secretary furnish for the Press such Report of the Proceedings as they may think proper.

On motion of Mr. Hilgard it was *Voted* that the meeting for Organization be with closed doors.

On motion of Mr. Hilgard, it was *Voted* that a committee of five members be appointed by the chairman to prepare & report upon (1) The form of a Diploma, (2) The Corporate Seal, (3) A stamp for Books and Property.   Messrs. F. A. P. Barnard, J. E. Hilgard, J. Saxton, L. M. Rutherfurd and J. P. Lesley were appointed.

It was voted that when we adjourn it be to meet at 8 o'clock this evening.

Adjourned.

<div align="right">ALEXIS CASWELL
Sec'y. pro tem.</div>

8 o'clock P. M.   April 22nd, 1863.

The chairman called the meeting to order agreeably to adjournment.

The Roll was called.   Shortly after Roll Call all the members present at the morning were believed to be present with the exception of James D. Dana, who had left N. Y. for New Haven.

The committee on organization reported through their chairman a series of Articles forty-five (45) in number which were recommended for adoption as the Laws of the Academy.

Prof. S. Alexander moved that the Report be printed and referred to a subsequent meeting for consideration.   —Motion lost.

On motion of Dr. B. A. Gould it was *Voted* that the Report be now taken up for consideration Article by Article in order.

Articles 1st, 2d, 3d, 4th, 5th, & 6th were adopted without material alteration.

Dr. Leidy moved that article 7th fixing the form of an oath of Allegiance to be taken by the members be amended by striking out the first part which refers to having borne arms against the Government or in any manner aided persons acting in hostility thereto.

After a somewhat protracted debate the motion was put and lost.

The article was then adopted as reported by the committee.

Articles 8th & 9th were adopted.

On motion of Prof. Frazer Article 10th referring to Elections was amended by substituting the word *"majority"* for the word *"plurality."*

Pending the consideration of the 10th Article the Academy adjourned to meet at 10 a. m. tomorrow.

ALEXIS CASWELL
Sec'y. pro tem.

Thursday April 23d, 10 A. M.

Pursuant to adjournment the meeting was called to order by the chairman.

The Roll was called and the following members answered to their names.

Agassiz	Hubbard	Guyot	Peirce
Bache	Leidy	Rogers, W. B.	Rogers, F.
Barnard, F. A. P.	Lesley	Saxton	Rogers, R. E.
Bartlett	Frazer	Winlock	
Caswell	Gibbs	Torrey	Coffin came in
Hall	Gilliss	Newberry	Hilgard after
Henry	Gould, B. A.	Newton	Strong Roll Call

Prof. Caswell moved a reconsideration of Article 7, fixing the form of the Oath of Allegiance with a view to offer an amendment which would limit its administration in its present form to the duration of the present Rebellion & thus obviate the main objection which had been urged against it.

After a brief debate the motion was withdrawn.

Prof. Peirce gave notice that he would at a later stage of the business offer a substitute for Article 7th.

Article 10th of the organic Laws which was under consideration at the time of adjournment last evening was resumed.

On motion of Prof. Frazer it was amended by substituting the word *"majority"* for the word *"plurality"* in the election of chairman of the classes.

On motion of Prof. Peirce the paragraph on the division of the members into sections was amended by adding the words following, viz., "By a special vote of the Academy a member may inscribe his name in a section of the class to which he does not belong."

Article 10th as amended was then adopted.

On motion of Prof. Bache the committee on Organization was reappointed and Article 11th was referred to them for revision.

Articles 12 to 23 inclusive were adopted.

On motion of Dr. Gould Article 24th was referred to the committee on Organization for revision.

Article 25th was adopted.

On motion of Prof. W. B. Rogers Article 26th fixing the requirement of memoirs and papers from the members was stricken out.

On motion of Dr. Gould it was *Voted* that the Academy adjourn at $3^{1}/_{2}$ p. m. to meet at 10 a. m. tomorrow.

The remaining articles numbered 27 to 45 inclusive (or in the printed copy 26 to 44) were adopted.

On motion of Prof. Bache, Prof. Winlock was added to the committee on organization.

At 4 p. m. the Academy adjourned to allow a short time for the committee on Organization to revise Articles 11 & 24.

At 4¹/₂ p. m. the meeting was again called to order by the chairman.

The committee reported articles 11 & 24 revised: and they were then adopted.

Prof. Peirce agreeably to previous notice moved a substitute for Article 7th (prescribing the form of the oath) in the words following viz., "All ordinary members of the Academy shall be citizens of the United States. Every member shall take the oath of Allegiance prescribed by the Senate of the United States for its own members; and in addition thereto, shall take an oath faithfully to discharge the duties of a member of the National Academy of Sciences to the best of his ability."

After a brief debate the substitute was adopted.

On motion of Dr. Gould Article 18th fixing the time of holding the stated meetings of the Academy was so amended that one of them shall be held on the *third day* of January (or if that be Sunday, on the Monday next following); and the other on the third Wednesday in August.

On motion of Prof. Bache the articles separately passed upon were provisionally adopted as a whole; and a committee of three was appointed to put them immediately in print with a view to a further revision on another day.

Committee—Messrs. Gibbs, Hilgard & Rutherfurd.

On motion of Prof. Frazer it was *Voted* that a committee of three be appointed by the chair to revise the style and arrangement of the articles; and report at the next stated meeting of the Academy.

Adjourned,

ALEXIS CASWELL

Sec'y. pro tem.

Friday 10 a. m. April 24th

Pursuant to adjournment the Academy was called to order by the chairman.

The minutes of the preceding meetings were read & corrected.

On the suggestion of the chairman Mr. F. Rogers was appointed assistant secretary pro tem.

On motion of Prof. Peirce it was *Voted* that in the minutes of the proceedings all titles of members shall be omitted, and the prefix "*Mr.*" used.

The committee on the Revision of the Laws was announced from the chair; viz., Mr. Frazer, Mr. Davis & Mr. Caswell.

On motion of Mr. Rutherfurd it was *Voted* that all the Articles of the Laws with the exception of Article 43th, relating the "Alteration of Laws" (on page 13 of the printed copy) be permanently adopted.

On motion of Mr. Frazer it was *Voted* that the Secretary be directed to call the Roll; and that each member be requested when his name is called to assign the *Class* and *Section* in which he wishes his name to be enrolled.

The roll was called, and the selections were as follows, viz.:

CLASS *A*. MATHEMATICS & PHYSICS.

Sec. 1. *Mathematics.* J. G. Barnard, Peirce, Strong and Winlock.

Sec. 2. *Physics.* Bache, Bartlett, F. A. P. Barnard, Henry and W. B. Rogers.

Sec. 3. *Astronomy, Geography* and *Geodesy.* Caswell, Coffin, Davis, Gilliss, Gould, Hubbard and Rutherfurd.

Sec. 4. *Mechanics.* Frazer, Hilgard, F. Rogers and Saxton.
Sec. 5. *Chemistry.* Gibbs, Silliman, B., Jr., and Torrey.

CLASS *B*. NATURAL HISTORY

Sec. 1. *Mineralogy* and *Geology.* Lesley and Newberry.
Sec. 2. *Zoology.* Agassiz.
Sec. 3. *Botany.*
Sec. 4. *Anatomy* and *Physiology.*
Sec. 5. *Ethnology.*

On motion of Mr. Gibbs it was *Voted* that a committee of two be appointed to arrange a book for the signatures of the members. Mr. Gibbs and Mr. W. B. Rogers were appointed said committee.

The Secretary then administered to the chairman the oath of allegiance to the Government, and of Fidelity to the Academy in the following words. (nomine mutate)

"I (A.B.) do solemnly affirm that I have never voluntarily borne arms against the United States since I have been a citizen thereof; that I have voluntarily given no aid, countenance, counsel or encouragement to persons engaged in armed hostility thereto; that I have neither sought nor expected to exercise the functions of any office whatever under any authority or pretended authority in hostility to the United States; that I have not yielded a voluntary support to any pretended government, authority, power or constitution within the United States, hostile or inimical thereto. And I do further affirm that to the best of my knowledge and ability, I will support and defend the Constitution of the United States against all enemies, foreign and domestic: that I will bear true faith and allegiance to the same; that I take this obligation freely, without any mental reservation, or purpose of evasion; and that I will well and faithfully discharge the duties of a member of the National Academy of Sciences. So help me God."

The chairman then administered the same oath to all the members, whose names are as follows.

Agassiz	Bache	Barnard, F. A. P.	Barnard, J. G.
Bartlett	Caswell	Coffin	Davis
Frazer	Gibbs	Gilliss	Gould, B. A.
Hilgard	Hubbard	Lesley	Newberry
Peirce	Rogers, F.	Rogers, W. B.	Rutherfurd
Saxton	Silliman, B., Jr.	Strong	Torrey
Winlock			

On motion of Mr. Frazer it was *Voted* that the Academy do now proceed to the election of officers.

On motion of Mr. Gibbs it was *Voted* that a committee of two be appointed to collect and count the votes. Messrs. Gibbs and F. A. P. Barnard were appointed the committee.

The chairman after a few remarks of warning and encouragement to the members before leaving the chair called for nominations for President of the Academy.

A. D. Bache was nominated.

The ballot was taken and on the Report of the tellers, *Alexander Dallas Bache* was declared by the chairman to be elected President of the Academy.

The chairman pro tem. then retired from the chair.

Mr. Caswell moved that Mr. Strong be appointed a committee to conduct the President elect to the chair. —Carried.

The President on taking the chair returned his thanks to the Academy for the honor they had done him, and proceeded with the business.

Nominations for Vice-President were called for.

James D. Dana was nominated.

The ballot was taken and *James D. Dana* was declared to be elected Vice-President.

For Foreign Secretary, Louis Agassiz was nominated. The ballot was taken and *Louis Agassiz* was declared to be elected.

For Home Secretary, Wolcott Gibbs was nominated. The ballot was taken and *Wolcott Gibbs* was declared to be elected. The Home Secretary requested the secretary pro tem. to retain his place till the close of the meeting.

For Treasurer, Fairman Rogers and L. M. Rutherfurd were nominated. The ballot was taken and *Fairman Rogers* was declared to be elected.

On proceeding to the election of Councillors, doubts were expressed whether it could with propriety be done at this time inasmuch as it was uncertain whether members not present would accept their appointment as Academicians under the Bill. Whereupon statements of members present showed that the following persons named in the Bill of Incorporation, but not present at this meeting, had signified their intention to accept their appointments as Academicians; viz., Mr. B. Silliman, Sen., Mr. J. Wyman, Mr. A. Gray, Mr. J. L. Leconte, Mr. G. Engelmann, Mr. W. Chauvenet, Mr. M. F. Longstreth and Mr. John Rodgers.

On motion of Mr. Frazer it was *Voted* that we proceed to the election of four members of the council.

Nominations for councillors were then called for, when the following names were announced.

Mr. Henry, Mr. Frazer, Mr. W. B. Rogers, Mr. Davis, Mr. Rutherfurd, Mr. Torrey, Mr. Lesley, Mr. Gilliss, Mr. Newberry and Mr. Gray.

Mr. Henry expressed a wish not to be elected, and hoped he might be allowed to withdraw his name.

After the first ballot, on Report of the tellers, Mr. *Charles H. Davis* was declared to be elected.

After the second ballot, Mr. *John Torrey* was declared to be elected.

After the third ballot, Mr. *L. M. Rutherfurd* was declared to be elected.

After the fourth ballot, Mr. *J. P. Lesley* was declared to be elected.

On motion of Mr. Frazer the committee on elections was discharged.

On motion of Mr. Davis it was *Voted* that the address with which the Hon. Henry Wilson inaugurated the first meeting of the National Academy of Sciences be entered upon the Journal in full; and that he be requested to furnish a copy for that purpose.

On motion of Mr. Frazer it was *Voted* that the thanks of the Academy be returned to the temporary chairman and secretary for the able manner in which they have discharged their duties.

Mr. B. Silliman, Jr., moved a recess for half an hour, or from 1:45 to 2:15 p. m. Carried; and the meeting adjourned.

At 2:15 the meeting was called to order by the President.

On motion of Mr. B. Silliman, Jr., a further recess was voted for a short time in order to give Class *A* an opportunity to organize.

At 2:45 p. m. the meeting was again called to order by the President.

Reports of the organization of classes were received, from which it appeared that the following class officers had been elected. Viz.—

Class A.   Benjamin Peirce, chairman.
           Benjamin A. Gould, secretary.

Class B.   Benjamin Silliman, Sen., chairman.
            J. S. Newberry, secretary.

The committee on the Diploma and Seal reported progress and also the following resolution which was passed, viz.,

Resolved that the committee on the Diploma and Seal be continued with instructions to report at the next stated meeting; and that Mr. F. Rogers and Mr. C. H. Davis be added to the committee.

On motion of Mr. Frazer it was *Voted* that in article 18 in the printed Laws (on pp. 6 & 7) another specification be added in the following words "12 rough minutes read for correction." And also that in No. 3 of the same article, the word *"correction"* be striken out and the word *"adoption"* inserted in its place.

On motion of Mr. Frazer it was *Voted* that the August stated meeting of this year be dispensed with.

On motion of Mr. Gibbs it was *Voted* that the thanks of the Academy be tendered to Chancellor Ferris for the use of Rooms in the University.

Mr. F. Rogers moved to amend Article 27th of the Printed Laws on p. 9 by adding at the beginning of the second paragraph the words *"short communications or,"* and by substituting for the word *"printed"* the words, *"published without delay."* Carried.

On motion of Mr. Frazer article 27 of the printed laws on p. 9 was amended by adding at the close of it the words following, viz., *"The Academy will not hold itself responsible for the opinions expressed in such papers."*

Mr. B. A. Gould moved the following resolutions.

Resolved that no more than ten Foreign Associates be elected at any one stated meeting. Carried.

On motion of Mr. B. A. Gould article 5th on page 2 of the printed Laws was amended by inserting at the beginning of the second paragraph the words, *"For ordinary members."*

Mr. Gould also moved that a committee of three be appointed to draft and present to the committee on the Revision for incorporation in their Report. *A Rule prescribing the mode of electing Foreign Associates.*—Carried.   Mr. Agassiz, Mr. Gould, and Mr. B. Silliman, Jr., were appointed said committee.

On motion of Mr. Frazer it was *Voted* that the President be requested to place his name on the committee of weights and measures when appointed.

At 4 o'clock p. m. on motion of Mr. Peirce, The Academy adjourned to meet in the city of Washington on the third day of January 1864.

ALEXIS CASWELL
Sec'y. pro tem.

Constitution and By-laws of the National Academy of Sciences

PREAMBLE

Empowered by the act of incorporation, adopted by Congress, and approved by the President of the United States on the 4th day of March, A.D. 1863, the National Academy of Sciences do enact the following constitution and by-laws:

ARTICLE I.

Of members.

SECTION 1.   The members of the Academy shall be designated as members, honorary members, and foreign associates.

Sec. 2. The Academy shall consist of the fifty members named in the act of incorporation, and of such others, citizens of the United States, as shall from time to time be elected to fill vacancies, in the manner hereinafter provided.

Sec. 3. Every member shall, upon his admission, take the oath of allegiance prescribed by the Senate of the United States for its own members, and, in addition thereto, an oath faithfully to discharge the duties of a member of the National Academy of Sciences to the best of his ability. He shall also subscribe the laws of the Academy.

Sec. 4. The members of the Academy shall be arranged in two classes, according to their special studies, viz.: A, the class of mathematics and physics, and B, the class of natural history. The corporate members may select the class in which they desire to be arranged.

Sec. 5. The members of the classes shall arrange themselves in sections by inscribing their names under one of the following heads: Class A, *mathematics and physics;* sections—1, mathematics; 2, physics; 3, astronomy, geography, and geodesy; 4, mechanics; 5, chemistry.

Class B, *natural history;* sections—1, mineralogy and geology; 2, zoology; 3, botany; 4, anatomy and physiology; 5, ethnology.

But the Academy retains the power of transferring a member from one section to another.

Sec. 6. A member may be elected an honorary member of any section by a vote of a majority of such section.

Sec. 7. The Academy may elect fifty foreign associates, who shall have the privilege of attending the meetings of the Academy and of reading and communicating papers to it, but shall take no part in its business, and shall not be subject to its assessments.

They shall be entitled to a copy of the publications of the Academy.

Article II.

Of the officers.

Sec. 1. The officers of the Academy shall be a president, a vice-president, a foreign secretary, a home secretary, and a treasurer; all of whom shall be elected for a term of six years by a majority of votes present at the first stated session after the expiration of the current terms, provided that existing officers retain their places until their successors are elected. In case of a vacancy, the election for six years shall be held in the same manner at the next stated session after the vacancy occurs.

Sec. 2. The officers of the classes shall be a chairman and a secretary, who shall be elected at each January session. The nominations shall be open, and a majority of votes shall be necessary to elect.

Sec. 3. The officers of the Academy and the chairmen of the classes, together with four members, two from each class, to be annually elected by the Academy, at the January session, by a plurality of the votes, shall constitute a council for the transaction of such business as may be assigned to them by the constitution or the Academy.

Sec. 4. The president of the Academy, or in case of his absence or inability to act, the vice-president, shall preside at the meetings of the Academy and of the council; shall name all committees, except such as are otherwise especially provided for; refer investigations required by the government of the United States to members specially conversant with the subject, and report thereon to the Academy at its next January session, and, with the council, shall direct the general business of the Academy.

It shall be competent for the president in special cases to call in the aid upon committees of experts or men of remarkable attainments, not members of the Academy.

Sec. 5. The foreign and home secretaries shall conduct the correspondence proper to their respective departments, advising with the president and council in cases of

doubt, and reporting their action to the Academy at its January session. It shall be the duty of the home secretary to give notice to the members of the place and time of all meetings, and to make known to the council all vacancies in the list of members.

The minutes of each session shall be duly engrossed before the next stated session, under the direction of the home secretary.

Sec. 6. The treasurer shall attend to all receipts and disbursements of the Academy, giving such bond and furnishing such vouchers as the council may require. He shall collect all dues from members, and keep a set of books showing a full account of receipts and disbursements. He shall present at each stated session a list of the members entitled to vote, and a general report at the January session. He shall be the custodian of the corporate seal of the Academy.

ARTICLE III.

Of the meetings.

Sec. 1. The Academy shall hold two stated sessions in each year: one in the city of Washington, on the third day of January (unless that day falls on Sunday, when the session shall be held on the succeeding Monday); and one in August, at such time and place as the Academy shall have determined upon, in private meeting, on the last day of the preceding January session.

Sec. 2. The names of the members present at each daily meeting shall be recorded in the minutes; and the members present at any meeting shall constitute a quorum for the transaction of business.

Sec. 3. Scientific meetings of the Academy, unless otherwise ordered by a majority of the members present, shall be open to the public; those for the transaction of business closed.

Sec. 4. The Academy may divide into classes for scientific or other business. In like manner, the classes may divide into sections.

Sec. 5. The classes shall meet during such periods of the stated meetings of the Academy as may be fixed by the Academy. Special meetings of a class may be called by the council at the request of five members of the class.

Sec. 6. The stated meetings of the council shall be held at the times of the stated or special meetings of the Academy. Special meetings shall be convened at the call of the president and two members of the council, or of four members of the council.

Sec. 7. No member who has not paid his dues shall take part in the business of the Academy.

ARTICLE IV.

Of elections, regulations, and expulsions.

Sec. 1. All elections shall be by ballot, unless otherwise ordered by this constitution; and each election shall be held separately.

Sec. 2. Whenever any election is to be held, the presiding officer shall name a committee to conduct it, to collect the votes, count them, and report the result to the Academy. The same law shall apply in the classes.

Sec. 3. Nominations for officers shall be made at the close of the first daily meeting of a stated session; and no candidate shall be voted for unless thus nominated.

Sec. 4. For election of members the council shall first decide the class in which the vacancy shall be filled. Each section of that class may then select one or more candidates after a discussion of their qualifications, and present their claims to the class, who shall select three to be presented in the order of their preference to the Academy; from these three the Academy shall elect by a majority of the members present. The member elect shall be assigned to the section in which he has been proposed. The Academy may nominate candidates in any section which fails to propose them for itself.

SEC. 5.   Every member elect shall accept his membership personally or in writing before the close of the next stated session after the date of his election.   Otherwise, on proof that the secretary has formally notified him of his election, his name shall not be entered on the roll of members.

SEC. 6.   Elections of foreign associates shall be conducted as follows:

Each section shall report to its class, nominating a candidate whose special researches need not belong within the province of the section, but must be comprised within the range of the class.

From these candidates each class shall select one name to be presented to the Academy, and from these two names the Academy, after full discussion, shall make the election, at such time as it may have previously appointed for the purpose.

SEC. 7.   A diploma, with the corporate seal of the Academy and the signatures of the officers, shall be sent by the appropriate secretary to each member on his acceptance of his membership.

SEC. 8.   Resignations shall be addressed to the president and acted on by the Academy.   No resignation of membership shall be accepted unless all dues have been paid.

SEC. 9.   Members resigning in good standing will retain an honorary membership; being admitted to the meetings of the Academy, but without taking part in the business. Honorary members will not be liable to assessment.

SEC. 10.   If any member be absent from four consecutive stated meetings of the Academy without communicating to the Academy a valid reason for his absence, his name shall be striken from the roll of members.

SEC. 11.   Members and officers habitually neglecting their duties shall be impeached by the council, and at once notified thereof in writing by the home secretary.

SEC. 12.   Impeachments of members or officers shall first be tried before the council, which may be convened specially for such purpose.   If it decides that the impeachment is proper, such impeachment shall be tried in private session before the Academy at its next stated meeting.

SEC. 13.   The expulsion of a member shall be formally and publicly announced by the president at the stated session during which expulsion shall take place.

ARTICLE V.

Of scientific communications, publications, and reports.

SEC. 1.   Papers on scientific subjects may be read at the meetings of the Academy, or of the classes or sections to which the subject belongs.

SEC. 2.   Any member of the Academy may read a paper from a person who is not a member, and shall not be considered responsible for the facts or opinions expressed by the author, but shall be held responsible for the propriety of the paper.

SEC. 3.   The Academy shall provide for the publication, under the direction of the council, of proceedings, memoirs, and reports.

SEC. 4.   Propositions for investigations or reports shall originate with the classes to which the subjects belong, and be by them submitted to the Academy for approval, except requests from the government of the United States, which shall be acted on by the president, who will in such cases report, if necessary, at once to the government, and to the Academy at its next stated meeting.

SEC. 5.   The judgment of the Academy shall be at all times at the disposition of the government upon any matter of science or art within the limits of the subjects embraced by it.

SEC. 6.   An annual report, to be presented to Congress, shall be prepared by the president, and before its presentation submitted by him first to the council, and afterwards to the Academy at its January meeting.

Sec. 7. Medals and prizes may be established, and the means of bestowing them accepted by the Academy upon the recommendation of the council, by whom all the necessary arrangements for their establishment and award shall be made:

Article VI.

Of the property of the Academy.

Sec. 1. All investments shall be made by the treasurer in the corporate name of the Academy in stocks of the United States.

Sec. 2. No contract shall be binding upon the Academy which has not been first approved by the council.

Sec. 3. The assessments required for the support of the Academy shall be fixed by the Academy on the recommendation of the council.

Article VII.

Of additions and amendments

Additions and amendments to the constitution shall be made only at a stated session of the Academy. Notice of a proposition for such a change may be given at any stated session, and shall be referred to the council, which may amend the proposition, and shall report thereon to the Academy at its next stated session, with a recommendation that it be accepted or rejected. Its report shall be considered by the Academy in committee of the whole, and immediately thereafter acted on. If the addition or amendment receive two-thirds of the votes present, it shall be declared adopted, and shall have the same force as the original law.

BY-LAWS.

Of the officers.

I. In the absence of the chairman or secretary of a class, a member shall be chosen to perform his duties temporarily, by a plurality of the *viva voce* votes, upon open nomination.

II. The accounts of the treasurer shall be referred to an auditing committee of three members, to be appointed by the Academy at the meeting at which the accounts are presented; which committee shall report before the close of that session, and shall then be discharged.

Of the meetings

III. A committee of arrangements, for each stated session of the Academy, of five members, shall be appointed by the president, the class secretaries to be *ex officio* two of the members of the committee. This committee shall meet not less than two weeks previous to each meeting. It shall be in session during the meetings to make arrangements for the reception of the members; to arrange the business of each day; to receive the titles of papers, reports, &c.; and to arrange the order of reading, and in general to attend to all business and scientific arrangements.

IV. At the meetings the order of business shall be as follows:

1. Chair taken by the president, or, in his absence, the vice-president.
2. Roll of members called by home secretary.
3. Report by treasurer of members entitled to vote.
4. Minutes of the preceding meeting read and approved.
5. Stated business.

6. Reports of president, secretaries, treasurer, classes, and committees.
7. Business from council.
8. Other business.
9. Communications from members.
10. Communications from persons not members.
11. Announcements of the death of members. Biographical notices read.
12. Rough minutes read for correction.

V. The rules of order of the Academy shall be those of the Senate of the United States, unless otherwise directed.

VI. It shall be in order for twelve members to require that any matter of business be discussed in committee of the whole for amendment; the vote upon amendments to be taken in the whole Academy; and the amended proposition or propositions to be similarly voted on.

VII. The scientific meetings shall be convened at twelve o'clock m., in order to allow time for the business meetings of the Academy, and for the meetings of classes, sections, and committees.

Of elections and obituaries.

VIII. No more than ten foreign associates shall be elected at any one stated session.

IX. The death of members shall be announced by the president on the last day of each stated session, when a member shall be selected by the Academy to furnish a biographical notice of the deceased at the next stated session. If such notice be not then furnished, another member shall be selected by the Academy in place of the first, and so on until the duty is performed.

X. The deaths of such eminent scientific men of the country as have taken place since the last session of the Academy shall be announced by the president. The names shall be selected by the council.

Of scientific communications, publications, and reports.

XI. An analysis of the memoirs and reports read in the meeting of the classes shall be given by the secretaries of the classes to the home secretary for publication in the proceedings of the Academy. For any failure in this duty, the delinquent officer shall be impeached by the home secretary.

XII. The secretaries shall receive memoirs at any time, and report the date of their reception at the next session. But no memoir shall be published unless it has been read before the Academy, class, or section, and ordered to be published by the Academy. Papers shall be published in the order in which they were registered, but papers which have not been sent to the secretary within a month from the time of their reading shall not be published without a special vote of the Academy

XIII. Memoirs shall date in the records of the Academy from the day of their presentation to the Academy, and this order of their presentation shall be that in which they were registered, unless changed by consent of the author.

XIV. The publication of any communication to which remonstrance is made by the section to which the subject belongs shall be suspended until a second time authorized by a vote of the Academy.

XV. Papers from persons not members, read before the Academy, classes, or sections, and intended for publication, shall be referred, at the meeting at which they are read, to a committee of members competent to judge whether the paper is worthy of publication. Such committees shall report to the Academy as early as practicable, and not later than the next stated session. If they do not then report they shall be discharged and the paper referred to another committee.

XVI.   Abstracts of papers published in the transactions of other societies or in journals may be communicated orally to the Academy; and if, on submitting any such communication to a committee, its publication be approved, it may be ordered for publication on a vote of the Academy.

XVII.   Short communications or abstracts of memoirs may be sent by any member to the home secretary, who shall, if requested by the author, without delay circulate them among the members.

XVIII.   An annual of the Academy shall be prepared by the secretaries, and published on the first day of each year.

XIX.   The printing of the Academy shall be under the charge of the secretaries and the treasurer, as a committee of publication, who shall report in relation thereto at each, January meeting of the Academy.

XX.   The annual report of the Academy may be accompanied by a memorial to Congress, in regard to such investigations and other subjects as may be deemed advisable, recommending appropriations therefor when necessary.

XXI.   The home secretary shall present to the council estimates for books and stationery, binding, &c., required for the use of the Academy.

Of the property of the Academy.

XXII.   The proper secretary shall acknowledge all donations made to the Academy, and shall report them at the next stated session.

XXIII.   The books, apparatus, archives, and other property of the Academy shall be deposited in some safe place in the city of Washington.   A list of the articles deposited shall be kept by the home secretary, who is authorized to employ a clerk to take charge of them.

XXIV.   A stamp corresponding to the corporate seal of the Academy shall be kept by the secretaries, who shall be responsible for the due marking of all books and other objects to which it is applicable.

Labels or other proper marks of similar device shall be placed upon objects not admitting of the stamp.

Of changes in the by-laws.

XXV.   Any by-law of the Academy may be amended or repealed on the written motion of any two members, signed by them, and presented at a stated session of the Academy; provided the same shall be approved by a majority of the members present at the next stated session.

From ANNUAL, NATIONAL ACADEMY OF SCIENCES, 1863-4-5-6

Address of the HON. HENRY WILSON

Delivered at the Opening of the First Session of the Academy, April 22, 1863.

Gentlemen:—I hold in my hand the Act, passed in the closing hours of the Thirty-seventh Congress, "To incorporate the National Academy of Sciences."   In compliance with many kind requests I am here to call the corporators to order.   In rising to perform this agreeable task, I crave for a moment your indulgence.

This Act, under which you have met to organize, incorporates in America, and for America, a National Institution, whose objects, ranging over the illimitable fields of science, are limited only by the wondrous capacities of the human intellect.   Such an institution has been for years in the thought and on the tongue of the devotees of science,

but its attainment seemed far in the future. Now it is an achieved fact. Our country has spoken it into being, in this "dark and troubled night" of its history, and commissioned you, gentlemen, to mould and fashion its organization, to infuse into it that vital and animating spirit that shall win in the boundless domains of science the glittering prizes of achievement that will gleam forever on the brow of the nation.

When, a few months ago, a gentleman, whose name is known and honored in both hemispheres, expressed to me the desire that an Academy of Physical Sciences should be founded in America, and that I would at least make the effort to obtain such an act of incorporation for the scientific men of the United States, I replied, that it seemed more fitting that some statesman of ripe scholarship should take the lead in securing such a measure, but that I felt confident I could prepare, introduce, and carry through Congress a measure so eminently calculated to advance the cause of science, and to reflect honor upon our country. I promptly assumed the responsibility, and with such aid and suggestions as I could obtain, I prepared, introduced, and by personal effort with members of both Houses of Congress, carried through this act of incorporation without even a division in either House.

The suggestion was sometimes made that the nation is engaged in a fearful struggle for existence, and the moment was not well chosen to press such a measure. But I thought otherwise. I thought it just the fitting time to act. I wanted the *savans* of the old world, as they turn their eyes hitherward, to see that amid the fire and blood of the most gigantic civil war in the annals of nations, the statesmen and people of the United States, in the calm confidence of assured power, are fostering the elevating, purifying, and consolidating institutions of religion and benevolence, literature, art and science. I wanted the men of Europe, who profess to see in America the failure of republican institutions, to realize that the people of the United States, while eliminating from their system that ever-disturbing element of discord, bequeathed to them by the colonial and commercial policy of England, are cherishing the institutions that elevate man and ennoble nations. The land resounds with the tread of armies, its bright waters are crimsoned, and its fields reddened with fraternal blood. Patriotism surely demands that we strive to make this now discordant, torn, and bleeding nation one and indivisible. The National Academy of Sciences will, I feel sure, be now and hereafter another element of power to keep in their orbits, around the great central sun of the Union, this constellation of sovereign commonwealths.

This act of incorporation may not be, is not, perfect. The task has been one of difficulty and delicacy. The number of members must be limited, while the most eminent men of science must be recognized, and sectional claims harmonized. If unintentional injustice has been done to any one, if mistakes have been made, time will, I trust, correct the injustice and the mistakes. Changes will surely come. "Death is in the world," and this original list of honored names will not remain long unbroken. If men of merit have been forgotten in this act of incorporation, the Academy should seize the first and every occasion to right the seeming wrong.

This Academy is destined, I trust, to live as long as the republic shall endure, and to bear upon its rolls the names of the *savans* of coming generations. Let it then advance high its standard. Let it be as inflexible as justice, and as uncompromising as truth. Let it speak with the authority of knowledge, that pretension may shrink abashed before it, and merit everywhere turn to it confident of recognition.

In the Providence of God, the Thirty-seventh Congress was summoned to the consideration of measures of transcendent magnitude. It enacted measures, empowering the government to raise hundreds of millions of dollars and millions of men, to protect the menaced life of the nation and preserve the vital spirit of freedom. It dealt with great questions of revenue and of finance. It obliterated an abhorrent system from the na-

tional capital, and engraved freedom upon every rood of the national territory. It consecrated the public domain to homesteads for the homeless and landless, and authorized the construction of a railway to unite the Atlantic and the Pacific seas. The enactment of this act to incorporate the Academy of Sciences was not the least in the long list of acts the Thirty-seventh Congress gave to the country, which will leave their impress upon the nation for ages yet to come. It was my fortune to take a humble part in these great measures of legislation. It is a source of profound gratification to me, that, amid the pressure of public affairs, I have been enabled to contribute something to found this Academy for the advancement of the physical sciences in America. It will ever be among my most cherished recollections, that I have been permitted through your courtesy to unite with you in organizing this National Academy, which, we fondly hope, will gather around it, in the centuries yet to come, the illustrious sons of genius and of learning, whose researches will enrich the sciences, and reflect unfading lustre upon the republic.

ERRATA

In the article "Absolute and Unconditional Convergence in Normed Linear Spaces" by A. Dvoretzky and C. A. Rogers, these PROCEEDINGS, **36**, 192–197 (1950), the following corrections should be made on page 197.

	for	read
Lines 4 and 9 from top:	Lemma 3	Theorem 5A
Formula (12):	n_n	n^n
Line 7 from bottom:	$S \subset K \subset \varphi$	$S \subset C \subset \varphi$

STRAIN SPECIFICITY AND PRODUCTION OF ANTIBIOTIC SUBSTANCES. IX. BACTERIOSTATIC AND BACTERICIDAL PROPERTIES OF NEOMYCIN AND DEVELOPMENT OF RESISTANT STRAINS*

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Communicated March 25, 1950

Introduction.—It was previously reported[1] that neomycin is highly effective against various bacteria, including both streptomycin-sensitive and -resistant strains. Further studies brought out the fact that when cultures of *Escherichia coli* were allowed to remain in contact with neomycin, no strains either highly resistant to or dependent on this antibiotic were produced. A certain degree of resistance to neomycin could be developed, but this took place rather slowly; the resistant cells also showed a marked tendency to become sensitive again. The growth of such resistant strains in the presence of neomycin in concentrations slightly lower than those required for inhibition was poor and was accompanied by marked changes in cell morphology.

The purpose of this investigation was threefold: first, to study the bactericidal and bacteriostatic action of neomycin, especially as compared to streptomycin; second, to measure the development of resistance to neomycin among sensitive bacteria; and third, to establish any possible synergistic effects of neomycin with streptomycin. The development of resistance of an organism to an antibiotic is of considerable importance in determining its usefulness as a potential chemotherapeutic agent. In measuring the potential synergistic combinations of the two antibiotics, the minimum concentrations which give the maximum antibacterial effect are first determined. The ratios of these antibiotics are then so adjusted that the addition of a minimum amount of neomycin would cause the optimum antibacterial action of streptomycin, without allowing the development of bacterial resistance to this antibiotic.

Experimental.—A laboratory strain of *E. coli* (No. 54) was used in these studies. This culture was initially sensitive to 3 μg./ml. of streptomycin

and to 0.5 u/ml. of neomycin. Higher concentrations of either antibiotic completely inhibited growth in nutrient broth or on nutrient agar. All cultures were incubated at 37°C.

Bacteriostatic and Bactericidal Properties of Neomycin.—As in the case with other antibiotics, the size of inoculum influenced considerably the amount of neomycin required for growth inhibition. When a 24-hr. agar slant culture of *E. coli* was washed with isotonic saline solution, and heavy suspensions of bacteria were added to nutrient agar containing different concentrations of the antibiotics, the amount of neomycin required for complete inhibition could be increased from 0.5 u/ml. to 5 u/ml. Similar concentrations of streptomycin had no effect upon such heavy inocula.

In comparing the bactericidal action of neomycin with streptomycin, suspensions of *E. coli* in 10-ml. portions of broth, to give approximately 180,000 cells per milliliter, were treated with varying concentrations of the two antibiotics. After 14 and 28 hours of incubation, aliquot portions were removed and plated out on nutrient agar (table 1). Complete in-

TABLE 1

Bᴀᴄᴛᴇʀɪᴏꜱᴛᴀᴛɪᴄ ᴀɴᴅ Bᴀᴄᴛᴇʀɪᴄɪᴅᴀʟ Aᴄᴛɪᴏɴ ᴏꜰ Nᴇᴏᴍʏᴄɪɴ ᴀɴᴅ Sᴛʀᴇᴘᴛᴏᴍʏᴄɪɴ

Inoculum—180,000 cells/ml. of broth. Plate method used for making counts of viable bacteria

	NUMBER OF VIABLE CELLS PER ML., AFTER INCUBATION FOR	
ANTIBIOTIC PER ML. OF BROTH	14 HRS.	28 HRS.
Control	800,000,000	700,000,000
Streptomycin, 1 μg.	340,000,000	330,000,000
Streptomycin, 5 μg.	80,000,000	40,000,000
Streptomycin, 20 μg.	0	0
Neomycin, 1 u	5,000	1,500,000
Neomycin, 5 u	0	0
Neomycin, 20 u	0	0

hibition of growth as well as a marked bactericidal effect took place with 20 μg./ml. of streptomycin. A concentration of 1 μg./ml. had only a slight inhibiting effect, and 5 μg./ml. had a marked bacteriostatic action but only a limited bactericidal effect. Neomycin was bacteriostatic and bactericidal in concentration of 5 u/ml. and with 1 u/ml., although with the latter not all the bacteria were killed. There was apparently some adaptation of the bacteria to the lowest concentration of neomycin, since there was an increase in the number of cells on further incubation of the cultures.

In the following experiment, turbidimetric measurements were made. A known suspension of bacteria was introduced into sterile Klett-Sommerson tubes. Known volumes of medium containing the antibiotic in different concentrations were added. A zero reading was taken immediately for each tube, and the tubes were incubated. The inoculum was obtained

by washing 24-hr. nutrient agar slants of *E. coli* with saline solution. The antibiotic solutions were heated at 70°C. for 10 minutes for sterilization.[2] Here again neomycin was found (table 2) to have a far greater bactericidal effect than streptomycin, 1.25 u/ml. of the first being comparable to 5 μg./ml. of the second.

A study was made next of the effect of neomycin upon a streptomycin-resistant strain of *E. coli*. The strain was repeatedly streaked on nutrient agar plates containing streptomycin and neomycin in different concentrations. It was found to give "normal" growth on all concentrations of the

TABLE 2

. EFFECT OF NEOMYCIN AND STREPTOMYCIN UPON THE GROWTH OF *E. coli*

Turbidimetric readings.   Original inoculum 2000 cells/ml. of broth.

ANTIBIOTIC PER ML. OF BROTH	INCREASE IN TURBIDITY OVER ZERO READINGS, AFTER INCUBATION IN			
	1 DAY	3 DAYS	7 DAYS	14 DAYS
Control	50	224	325	435
Streptomycin, 1.25 μg.	18	79	119	220
Streptomycin, 2.5 μg.	22	78	106	206
Streptomycin, 5 μg.	0	0	0	0
Streptomycin, 10 μg.	0	0	0	0
Neomycin, 1.25 u	0	0	0	0
Neomycin, 2.5 u	0	0	0	0
Neomycin, 5 u	0	0	0	0

TABLE 3

EFFECT OF NEOMYCIN UPON STREPTOMYCIN-RESISTANT STRAIN OF *E. coli*

Turbidimetric readings.   Original inoculum 1000 cells/ml. of broth.

ANTIBIOTIC PER ML. OF BROTH	INCREASE IN TURBIDITY OVER ZERO READING, AFTER INCUBATION IN	
	5 DAYS	12 DAYS
Control	162	284
Streptomycin, 5 μg.	141	269
Streptomycin, 10 μg.	162	365
Streptomycin, 100 μg.	146	275
Neomycin, 1.25 u	0	0
Neomycin, 2.5 u	0	0
Neomycin, 5 u	0	0

first and was sensitive to 1.25 u/ml. of the second.   A suspension of a 24-hr. nutrient agar slant was used to inoculate a series of Klett tubes.   The inoculum was so adjusted as to give 1000 cells/ml. of the final medium. Growth was more rapid and much heavier in the tubes containing streptomycin than in the control tube (table 3), possibly because of the presence of streptomycin-dependent cells in the culture.   The lowest concentration of neomycin, 1.25 u/ml., completely inhibited growth of this strain.   These results tend to show that streptomycin-resistant strains of *E. coli* do not develop simultaneous resistance to neomycin.

Development of Resistance by E. coli to Neomycin.—The *E. coli* culture used in these experiments was inhibited by 1 u/ml. of neomycin or less, the size of inoculum being of great importance in this connection, as shown in table 4. On several occasions, a few colonies appeared on the plates containing 3 to 5 u/ml. of neomycin, and even on a 10 u/ml. plate. The cultures obtained from these colonies died, however, after passage through liquid medium containing 12 u/ml. of neomycin. The only time when such colonies grew in neomycin-containing media in concentrations exceeding 2 u/ml. was when the plates were streaked with a heavy cell suspension. No growth was ever observed on dilution plates or in liquid media containing more than 2.5 u/ml. of neomycin.

A streptomycin-dependent strain of *E. coli* failed to grow in the absence of streptomycin or in the presence of neomycin. A streptomycin-sensitive strain of *B. subtilis* was completely inhibited by 0.2 u/ml. of neomycin.

TABLE 4

GROWTH OF *E. coli* IN VARIOUS CONCENTRATIONS OF NEOMYCIN AS AFFECTED BY SIZE OF INOCULUM

NUMBER OF CELLS IN INOCULUM	NEOMYCIN (u/ml.)						
	0	0.2	0.4	0.6	0.8	1.0	1.8
				Turbidity[a]			
63.7 × 10	30	19	0	0	0	0	0
63.7 × 10²	34	22	0	0	0	0	0
63.7 × 10³	35	21	20[b]	0	0	0	0
63.7 × 10⁴	30	18	30[b]	0[b]	0	0	0
63.7 × 10⁵	31	18	24	28[c]	22[b]	0	0
63.7 × 10⁶	30	23	19	20	16	0	0

[a] 100 per cent transmission. Readings represent averages of three tubes at 144 hrs incubation except where noted

[b] Average turbidity of two tubes only

[c] Turbidity of one tube only

A neomycin-resistant strain of *Mycobacterium* 607 grew at fairly high concentrations of neomycin; it showed also sporadic resistance to streptomycin, although in most cases it was completely inhibited by 1 μg./ml.

In a natural population, a few cells resistant to neomycin were found occasionally (table 5). The most resistant cells were grown in the presence of increasingly higher concentrations of this antibiotic. Considerable difficulty was encountered, however, since such resistant cultures again became readily sensitive to neomycin. When cultures were made resistant to concentrations of neomycin as high as 70 u/ml. by gradual transfer, they failed, after several transfers, to make further growth or grew only in media containing no neomycin.

A long period of incubation was sometimes required for growth of cultures in neomycin-containing media. Growth often failed to appear in cultures incubated at 28°C. Yeast extract agar media gave growth in some instances when none was obtained on beef extract media.

A strain of *E. coli* was finally developed which was resistant to 100 u/ml. of neomycin. Fifteen consecutive transfers were required for this purpose, using increasing concentrations of the antibiotic. Agar slants containing 50, 60 and 70 units/ml. of neomycin were streaked with this strain. Growth was obtained after one day's incubation at 37°C. The slants were stored in the icebox, together with cultures growing in media containing no neomycin. After four weeks, all the cultures were tested. Only those which grew on media containing no neomycin contained viable cells; this was also true of a few slants containing less than 30 units/ml. neomycin. The cultures growing in neomycin-free media were still resistant to 40 u/ml. *E. coli* can thus be made resistant to neomycin in concentrations less than 100 u/ml., but the strain dies out rapidly or becomes sensitive when exposed for prolonged periods to neomycin. The strain can retain its resistance to neomycin when kept on neomycin-free media.

TABLE 5

DEVELOPMENT OF NEOMYCIN-RESISTANT CELLS OF *E. coli* IN THE ABSENCE OF NEOMYCIN

INCUBATION, HRS	TOTAL NUMBER OF CELLS/ML.	CELLS RESISTANT TO 5 UNITS NEOMYCIN/ML AGAR
0	3,480,000	1
4	13,800,000	22
8	116,000,000	40
12	184,000,000	77
24	310,000,000	48
48	392,000,000	8
72	363,000,000	8
96	367,000,000	2
144	322,000,000	4
219	327,000,000	5

The rate of growth and the number of cells were less in the neomycin-resistant than in the sensitive cultures, even upon prolonged incubation. Growth on solid media was always in the form of very small, moist, glistening colonies; large colonies or heavy growth was seldom obtained, such growth being sensitive to neomycin. Growth in liquid media showed a fine granular dispersion rather than the homogeneous turbidity of young cultures of sensitive strains. Microscopic appearance revealed cells normal in size, but darker in staining, during the first 20 hrs. of incubation; after that, the cells usually became elongated and had a granular appearance. Those slants which initially grew in the presence of neomycin and then died out showed a lysed appearance, the presence of growth being almost indiscernible to the naked eye.

In the early stages of the development of neomycin resistance, no increase in resistance to streptomycin was noted. When the neomycin-resistant strain was kept on neomycin-free media in the icebox for four weeks

and then tested, it was found to show an increase in resistance to 75 μg./ml. of streptomycin. This points to the possibility of a certain amount of overlapping in the mode of action of the two antibiotics.

The Synergistic Effects of Neomycin and Streptomycin.—For the study of the synergistic effects of the two antibiotics on *E. coli*, both turbidimetric measurements and determination of numbers of viable cells by the agar plate method were used. Certain combinations of neomycin with streptomycin showed marked bacteriostatic and bactericidal effects, which were considerably greater than the corresponding concentrations of either antibiotic alone. This was even true when the ratio of streptomycin to neomycin was 15:1 and higher, complete inhibitions of growth taking place with ratios of 6:1.

A combination of 1 μg./ml. of streptomycin and 0.2 u/ml. of neomycin exerted a bactericidal effect, though 3.2 μg./ml. of streptomycin alone or 0.2 u/ml. of neomycin alone showed relatively little action. This is brought out in table 6. The agar plate method makes possible a further insight into

TABLE 6

SYNERGISTIC ACTION OF NEOMYCIN AND STREPTOMYCIN UPON SMALL NUMBERS OF *E. coli* CELLS

Turbidimetric readings. Inoculum 1750 cells/ml. of broth

ANTIBIOTIC PER 1 ML. OF BROTH	INCREASE IN TURBIDITY OVER ZERO READING, AFTER INCUBATION IN		
	2 DAYS	4 DAYS	7 DAYS
Control	205	312	372
Streptomycin, 1.2 μg.	59	88	133
Streptomycin, 3.2 μg	45	60	75
Neomycin, 0.2 u	42	52	90
Neomycin, 0.5 u	0	0	0
1 μg. streptomycin + 0.2 u neomycin	0	0	0
3 μg. streptomycin + 0.2 u neomycin	0	0	0

the inhibiting action of the two antibiotics upon the growth of *E. coli:* A combination of 2.5 μg./ml. streptomycin plus 2.5 u/ml. of neomycin gave nearly complete inhibition, whereas heavy growth was obtained on the plates containing either of these antibiotics alone, as well as on the plates containing 5 μg./ml. of streptomycin (table 7).

To test the possible development of resistance of the surviving cells, transfers were made from the few colonies that appeared on the plates which contained both antibiotics into nutrient broth to which varying concentrations of streptomycin, neomycin, and combinations of both were added. No resistance to neomycin greater than one unit was found. The colonies isolated from the plates containing both antibiotics showed no great resistance to streptomycin either.

Summary.—1. The bacteriostatic and bactericidal properties of neo-
mycin were compared with those of streptomycin. Growth of *E. coli* in
nutrient broth was measured by the turbidimetric and plating procedures.

2. The bactericidal action of neomycin was much greater than that of
streptomycin. Concentrations as low as 1 u/ml. of neomycin greatly in-
hibited growth of *E. coli* in heavy suspensions, whereas similar concen-
trations of streptomycin had little effect. When the inoculum was so ad-
justed that the final medium contained a few hundred or thousand cells
per milliliter, concentrations of neomycin as low as 0.5 u/ml. inhibited
growth completely.

3. A streptomycin-resistant strain of *E. coli* showed no increase in re-
sistance to neomycin. A streptomycin-dependent strain of *E. coli* failed to
grow in the presence of neomycin.

4. *Mycobacterium* 607 was inhibited by 1 u/ml. of neomycin. A strain
of this organism made neomycin-resistant was usually inhibited com-

TABLE 7

SYNERGISTIC EFFECTS OF NEOMYCIN AND STREPTOMYCIN UPON *E. coli*, AS MEASURED
BY AGAR PLATE METHOD

ANTIBIOTIC PER ML. OF AGAR	NUMBER OF COLONIES PER PLATE
Control	> 1000
Streptomycin, 2.5 μg.	> 300
Steptomycin, 5 μg.	40–> 300
Streptomycin, 10 μg.	0
Neomycin, 1.25 u	> 300
Neomycin, 2.5 u	33–119
Neomycin, 5 u	0
2.5 μg. streptomycin + 2.5 u neomycin	0*
3 μg. streptomycin + 2 u neomycin	0*
4 μg. streptomycin + 1 u neomycin	0–5*

* A single colony found on 2 out of 5 plates.
* A total of 7 colonies on 5 plates.

pletely by 1 μg./ml. of streptomycin, but in several instances colonies were
found on plates containing 50 μg./ml.; this strain remained resistant to
high concentrations of neomycin for several months.

5. A strain of *E. coli* originally sensitive to neomycin was made resis-
tant to 100 u/ml. of neomycin. Such cultures usually died out or became
sensitive again after several transfers. A few cultures retained their re-
sistant properties to a greater extent when grown in media containing no
neomycin. The incubation time of neomycin-resistant cultures was longer
than that of sensitive cultures.

6. The neomycin-resistant strain of *E. coli* grew on solid media in the
form of small, glistening colonies; in liquid media, growth appeared as a
granular suspension. Dead colonies had a lysed appearance.

7. Certain combinations of neomycin and streptomycin showed considerably greater bacteriostatic and bactericidal effects than the corresponding concentrations of either antibiotic alone or concentrations of streptomycin equal to the total number of units of both antibiotics. Such antibacterial effects were never greater, however, than concentrations of neomycin equal to the total units of the two antibiotics. Very small amounts of neomycin in synergistic mixtures with streptomycin had a much greater effect than streptomycin alone.

8. No marked increase in the resistance of *E. coli* cells which survived exposure to neomycin and streptomycin was observed toward either antibiotic or combinations of the two.

9. The neomycin-resistant strain of *E. coli* showed some resistance to streptomycin after several weeks on media containing neither antibiotic.

10. Neomycin appears to be about four times as active, on a unit basis, as streptomycin against many bacteria. In view of the fact that the most potent neomycin preparations so far obtained have 250 units per milligram, the antibacterial potency of the two antibiotics is about similar on a gram basis.

Acknowledgment.—The authors wish to express their appreciation to Dr. Warren P. Iverson, formerly of this department, who carried out preliminary studies on this problem.

ANTIBIOTIC SUBSTANCES FROM BASIDIOMYCETES. VII. CLITOCYBE ILLUDENS*

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Clitocybe illudens[1] was reported earlier from this laboratory[2] to evidence antibacterial activity. In later tests the antibacterial action of this fungus was found to be substantially greater on a corn steep agar medium than on the original thiamine-peptone agar or on a potato-dextrose agar. Grown on corn steep agar and tested by the agar disc method,[2, 3] zones of inhibition up to 25 mm. in diameter were observed for *Staphylococcus aureus*

and up to 40 mm. in diameter for *Mycobacterium smegma.*   There was little or no effect on *Escherichia coli.*

Culture liquids with antibacterial activity were produced by growing the fungus at 25°C. on a corn steep medium in Fernbach flasks as previously described.[4]   After three to four weeks the mycelial mats covered the surface and the culture liquids had an activity of from 64 to 256 dilution units per ml. against *Staph. aureus, Klebsiella pneumoniae* and *Myco. smegma.* Little inhibitory action was noted on *Bacillus mycoides, Bacillus subtilis, E. coli* or *Pseudomonas aeruginosa.*   It was possible to obtain active liquid two weeks after reflooding such mats with fresh corn steep medium.   Mats were successfully reflooded several successive times.

Isolation of Crystalline Substances.—Two antibacterial substances and one substance inactive for the organisms tested were isolated in crystalline form from culture liquids of *C. illudens.*

Extraction of the active material from the culture liquid with organic solvents was incomplete.   However, the active material was adsorbed practically quantitatively by charcoal from which it could be eluted by aqueous acetone.

Bioassay of the material extracted by organic solvents and that left in the aqueous phase suggested the presence of more than one active component.   Thus after extraction of culture liquid with chloroform the activity for *Myco. smegma,* compared to that for *Staph. aureus,* was increased in the organic solvent and decreased in the aqueous phase.   By countercurrent distribution between chloroform and water, of the material eluted from the charcoal, three distinct components were separated.

Batches of culture liquid at their original pH of from 4.2 to 4.5 were stirred for an hour with 20 g. of Norit A (Pfanstiehl) per liter, using an efficient mechanical stirrer.   The solution was usually kept in a cold room at 8° to 10°C. overnight, and the charcoal allowed to settle.   After the charcoal had been filtered off by suction, through a thin layer of celite, a perfectly clear solution was obtained which had only a small percentage of the original activity; this was discarded.   The Norit A, on which the active material was adsorbed, was stirred for three half-hour periods with 80 per cent acetone, the first time with 10 per cent of the volume of culture liquid· used, then twice more with 5 per cent each time.   The eluate was concentrated under reduced pressure, in a water bath at about 60°C. to remove the acetone, leaving about a liter of aqueous concentrate.   This contained essentially all the active material originally present in the culture liquid. Fractionation was accomplished by countercurrent distribution.   The distribution was carried out in ten funnels, using equal volumes of chloroform and water.

By concentration of the extract to a small volume under reduced pressure, and finally to a sirup under a stream of nitrogen, crystalline ma-

terial was usually obtained from "Chloroform 1" (the first chloroform extract which passed through the series of funnels) and sometimes also from "Chloroform 2." This material was over a hundred times as active against *Myco. smegma* as against *Staph. aureus;* it is designated *illudin M.* Chloroforms 4 to 10 on evaporation yielded inactive crystalline material.

"Water 1" (the original eluate after being washed with ten portions of chloroform) and "water 10" (the tenth water washing of the chloroform) were of low antibacterial potency and accounted for only a small fraction of the activity of the original culture liquid. These extracts were discarded. Waters 2 to 9 were extracted with two and one-half times their volume of ethyl acetate in five equal portions. On evaporation of the ethyl acetate, a crystalline compound highly active against both *Staph. aureus* and *Myco. smegma* was obtained. This substance is referred to as *illudin S.*

The yield of crystalline illudin S per liter of culture fluid (average of ten batches) was 0.33 g.; of crystalline illudin M, 0.04 g. About one-third of the activity of the original culture liquid against *Staph. aureus* was recovered in the crystalline material and about one-half of the activity against *Myco. smegma*. The average results per liter for ten batches of culture liquid are given below:

	WEIGHT, G.	*Staph. aureus* DILUTION UNITS	*Myco. smegma* DILUTION, UNITS
Illudin S	0 33	75,460	20,650
Illudin M	0.04	260	30,140
Total	0.37	75,720	50,790
Culture liquid	. . .	230,000	101,000

By redistribution of mother liquors from which crystals had been obtained, more of each of the compounds was crystallized. The chloroform mother liquors yielded varying amounts of crystals of the inactive compound in addition to illudin M. Average results of redistribution of three batches of chloroform mother liquors and four batches of ethyl acetate mother liquors are given below:

	WEIGHT, G.	ACTIVITY	
		Staph. aureus DILUTION UNITS	*Myco. smegma* DILUTION UNITS
No. 1, Solids in CHCl₄ mother liquors	29.00	238,000	4,500,000
Illudin M from No. 1	1.01	6,200	907,000
Inactive crystals from No. 1	0.50	. . .	. . .
No. 2, Solids in ethyl acetate mother liquors	30.00	3,900,000	1,400,000
Illudin S from No. 2	5.51	718,000	804,000
Total illudin M and illudin S	6.52	724,200	1,711,000

If the additional material obtained by redistribution is taken into account the percentage of the activity of the original culture liquid against

Staph. aureus which was recovered in crystalline form was about 45 per cent; against *Myco. smegma*, about 80 per cent.   While it is possible that other antibiotic substances may have been present, it is believed that illudin M and illudin S account for the major part of the activity of the culture liquids.

Chemical Characteristics of Illudin M and Illudin S.—The antibacterial activity of the culture liquid, or of the active crystalline compounds dissolved in water, was not reduced by bringing the solution to a quick boil. This method of sterilization was used in preparing samples for bioassay. The activity of the culture liquid was not affected by incubation for one hour at 37°C. and pH levels of from 3 to 11.   While no systematic studies of stability have been made, it has been observed that the crystalline compounds are unstable under somewhat more stringent conditions than those mentioned.

Both active compounds are fairly soluble in organic solvents, less so in water.   Illudin M is soluble in water to the extent of at least 1 mg. per ml.; illudin S is somewhat more soluble.

The three crystalline compounds have been further purified and characterized; the data given below suggest that they are probably of related molecular structure.   The molecular formula of illudin M differs from that of illudin S by the elements of one molecule of water.

Compound	Illudin M	Illudin S	Inactive crystals
Recrystallized from	Aqueous ethanol	Acetone	Chloroform
Melting point (uncorr.)	130–131°	124–125°	72–74°
Absorption maxima (mμ) in 95% ethanol	230, 320	235, 328	None between 215 and 360
$(\alpha)_D^0$ in absolute ethanol	−126	−165	−107
Analytical values, C, H[5]	C, 72.81	67.78	60.39
	H, 8.05	8.16	8.29
Empirical formula, and calculated C, H	$C_{15}H_{20}O_3$	$C_{15}H_{22}O_4$	$C_5H_6O_2$
	C, 72.53	67.62	59.98
	H, 8.12	8.33	8.06
Molecular weight (found)[5]	241	264	204
Molecular formula	$C_{15}H_{20}O_3$ (mol. wt. 248)	$C_{15}H_{22}O_4$ (mol. wt. 266)	$C_{10}H_{16}O_4$ (mol. wt. 200)[5] or $C_{15}H_{24}O_5$ (mol. wt. 300)

All three compounds are neutral.   A further similarity is their behavior in acid solution, in which characteristic changes occur in their absorption spectra.   The products resulting from this change, and their possible bearing on the structure of the antibiotic compounds, will be reported separately.

Antibacterial and Antifungal Activity.—The potency of the active crystalline compounds, after recrystallization, was determined for a

number of bacteria by serial dilution[7] and is given in the following table. The activity is expressed as the minimum inhibitory concentration in μg. per ml. (*p* = partial inhibition)

Bacterium	Illudin S	Illudin M
Bacillus mycoides	> 500	> 500
Bacillus subtilis	31 (16*p*)	500
Escherichia coli	> 500	> 500
Klebsiella pneumoniae	4	16 (8*p*)
Mycobacterium smegma	4 (2*p*)	1 (0.5*p*)
Mycobacterium tuberculosis[8] H37R	6	1
Pseudomonas aeruginosa	500 (125*p*)	> 500
Staphylococcus aureus	4	250

The potency of the antibacterial compounds against *Staph. aureus*, *Myco. smegma* and *K. pneumoniae* was not reduced after incubation at 37°C. for three hours with 5 per cent human blood in 0.9 per cent saline with beef extract.

The antifungal activity of illudin S and illudin M was measured by serial dilution in a peptone medium at pH 6. Spore suspensions of the fungi were used as inoculum.[9] *Trichophyton* was incubated at 30°C.; the others at 25°C. The fungi tested included *Aspergillus niger*, *Chaetomium globosum* (USDA 1042.4), *Gliomastix convoluta* (PQMD4c), *Memnoniella echinata* (PQMD1c), *Myrothecium verrucaria* (USDA 1334.2), *Penicillium notatum*, *Phycomyces Blakesleeanus* (plus strain), *Saccharomyces cerevisiae*, *Stemphylium consortiale* (PQMD41b), and *Trichophyton mentagrophytes*. As much as 250 μg. per ml. of illudin S did not inhibit the growth of these fungi.

The antifungal activity of illudin M was somewhat greater, though the difference in response between fungi was marked. A wide range of partial inhibition (*p*) was noted for some species. The minimum inhibitory concentration of illudin M in μg. per ml. was 16 for *Memnoniella echinata*, 16 (2*p*) for *Penicillium notatum*, 32 (1*p*) for *Chaetomium globosum*, 64 (32*p*) for *Trichophyton mentagrophytes*, 125 (8*p*) for *Myrothecium verrucaria*, 250 (32*p*) for *Aspergillus niger*, 250*p* to 32*p* for *Stemphylium consortiale*, 250*p* to 125*p* for *Saccharomyces cerevisiae*, 250*p* for *Phycomyces Blakesleeanus* and more than 250 for *Gliomastix convoluta*.

Toxicity.—In preliminary tests, using Carworth male white mice weighing on an average, 16 g., all mice were killed by illudin S at 15.6 mg. per kilo in from seven to twenty-two hours, and by illudin M within forty-four hours. Four groups of five mice each were used in the tests: one control group, and three groups receiving 0.25, 0.50 and 1.0 mg. per mouse, respectively. The test compound was dissolved in 0.5 ml. of 0.9 per cent saline solution, and injected into a tail vein. The controls received only saline solution.

Persons handling *Clitocybe illudens* or its products showed some sensitivity on contact, which manifested itself as a dermatitis of varying degrees of severity.

Hollande obtained an antibacterial substance, clitocybine, from the sporophores and mycelium of *Clitocybe gigantea* (*Fr. ex. Sow.*) *Quel. var. candida* (*Bres.*) *Heim*. The chemical properties given for clitocybine[10] distinguish it from illudin M and illudin S.

The sporophores of *Clitocybe illudens* are poisonous when eaten.[11]   Clark and Smith[12] concluded from chemical and biological evidence that *C. illudens* had a muscarin-like effect.   They were able to overcome the toxic action on frogs' hearts by atropin.   Illudin M and S do not appear to be related to muscarin; it is, however, possible that the compounds found in sporophores of the fungus differ from those produced in the culture liquid by the mycelium.

[1] We are indebted to Ross W. Davidson, Division of Forest Pathology, U. S. Department of Agriculture for the culture of *Clitocybe illudens*, No. 72027-S in his collection.

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[3] Hervey, A., *Ibid.*, **74**, 476–503 (1947).

[4] Robbins, W. J., Kavanagh, F., and Hervey, A., these PROCEEDINGS, **33**, 171–176 (1947).

[5] The carbon and hydrogen analyses and molecular weight determinations were carried out by Joseph Alicino, Metuchen, N. J.

[6] The molecular weight of this compound is uncertain since it shows some signs of decomposition during the camphor melt.

[7] Kavanagh, F., *Bull. Torrey Botan. Club*, **74**, 303–320 (1947).

[8] Determinations for *M. tuberculosis* were made through the courtesy of Dr. Walsh McDermott and Dr. W. C. Robbins of the Cornell Medical College.

[9] Kavanagh, F., Hervey, A., and Robbins, W. J., these PROCEEDINGS, **35**, 343–349 (1949).

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[12] Clark, Ernest D., and Smith, Clayton, S., *Mycol.*, **5**, 222–232 (1913).

UNILATERAL NUCLEAR MIGRATION AND THE INTERACTIONS OF HAPLOID MYCELIA IN THE FUNGUS CYATHUS STER-COREUS

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The researches of Kniep[1] in 1919 established the fact that, among certain Basidiomycetes, sexuality is governed by two pairs of independently segregating factors. In many of these fungi, the mycelium which develops from a single spore is haploid and when two compatible haploid mycelia grow together and fuse, diploid or dicaryotic mycelium is formed. Compatibility of haploid mycelia is determined, in the so-called "tetrapolar" species, by two pairs of independently segregating factors, $A-a$ and $B-b$. Haploid mycelia AB, ab, Ab and aB are sexually compatible only in combinations which provide the full set of factors, $AaBb$.[2]

Many fungi are now known to exhibit this type of sexuality. The method by which diploid mycelia are produced following fusion of compatible haploid mycelia was first demonstrated by Buller.[3] He was able to show that when two compatible haploid mycelia fuse, nuclei migrate from each mycelium into the other and, as a result of this nuclear migration, both haploid mycelia become converted into diploid mycelia. Buller further showed that in this process of "diploidization" the rate of nuclear migration greatly exceeds the normal growth rate of either the haploid or the diploid mycelium.

Despite Buller's experiments and other subsequent studies there is, as yet, little knowledge of the mechanism of nuclear migration in the higher fungi. It is not known, for instance, what role is played by the two pairs of compatibility factors in the process of diploidization. The interactions of the mycelia of certain fungi would seem to suggest that there may be a different function for each of the two pairs of factors. For example, the "barrage" phenomenon studied by Vandendries and Brodie[4] in *Lenzites betulina* occurs only when two incompatible haploid mycelia are paired which contain different members of one pair of factors, in this species designated as $B-b$, and the same A or a factor.

Experimenting with *Coprinus lagopus*, Buller[5] showed that each haploid of a compatible pair undergoes diploidization. The nuclei migrate from both haploid mycelia into the mycelium of the partner. Certain exceptions to this type of regular diploidization have been observed. Dickson[6] reported that in some compatible pairings of haploid mycelia of *Coprinus sphaerosporus* only one of the haploids became diploid.

Attention was drawn by Brodie[6] in 1948 to this kind of diploidization in

Cyathus stercoreus. In some pairings of haploid mycelia ($AB \times ab$) the AB became diploid; the ab remained haploid. The term "unilateral diploidization" was applied by Brodie to the phenomenon, in reference to the migration of nuclei in one direction only (from ab mycelium into AB) to distinguish the situation from that commonly observed in which nuclei migrate from both mycelia of the compatible pair.

The present writer, in 1947, began an investigation of unilateral diploidization in the hope of throwing more light on the general problem of nuclear migration and the processes controlled by the mating type factors (see Brodie[6]). The present paper is a preliminary résumé of the chief results obtained.

Forty monospore cultures were obtained in 1949 from spores taken from one peridiole of *Cyathus stercoreus* grown in laboratory culture by Dr. Brodie.[6] Although similar results have been obtained from three other series of isolates (152 in all) from different fruit bodies, the observations recorded here and the conclusions drawn are based entirely on the study of the 1949 series. This is done because the assignment of genetic factors for mating types based on the results of pairings is arbitrary, and since it was not possible to keep cultures in a healthy condition for more than one year, it was not possible to cross different series as would be necessary in order to prove that mating types of different series were strictly comparable.

After being grown for three weeks, the haploid mycelia were paired in tubes in all possible combinations. Pairings on agar plates were made when it was necessary to study the interaction of mycelia more closely. The formation of diploid mycelium bearing true clamp connections was used as the criterion of sexual compatibility. From the results, the haploid mycelia were assigned to four mating types: AB, ab, Ab and aB.

When haploid mycelia were paired on agar plates, several types of interaction were observed, as shown in table 1. We may now consider each of these types of reaction in turn.

I. Combinations of Compatible Mycelia ($AB \times ab$ and $Ab \times aB$).—As shown in table 1, the diploidization in the majority of pairings of compatible mycelia was of the "regular" type. When two compatible haploids came into contact, hyphal fusions took place in the region of contact and diploid mycelium gradually appeared around the periphery of both haploid mycelia. Within 5–7 days both mycelia had become completely diploid.

Unilateral diploidization was found in some pairings representing each of the compatible combinations of mycelia. In unilateral pairings of $AB \times ab$ (pairings in which unilateral diploidization was manifest), the AB mycelium was always the acceptor of nuclei, i.e., it became diploid; the ab mycelium was always the donor of nuclei and remained haploid. Uni-

TABLE 1

INTERACTIONS OF FORTY MONOSPOROUS MYCELIA OF *Cyathus stercoreus* WHEN PAIRED IN ALL POSSIBLE COMBINATIONS

REACTION TYPE	GENOTYPES OF PAIRED MYCELIA	REACTION	TOTAL COMBINATIONS	NUMBER GIVING VISIBLE REACTION	NUCLEAR MIGRATION		
					REGULAR	UNILATERAL	NUMBER TESTED
I	(Compatible) $AB \times ab$	Formation of diploid mycelium	100	100	61	39	100
	$Ab \times AB$	Formation of diploid mycelium	66	66	14	6	20

REACTION TYPE	GENOTYPES OF PAIRED MYCELIA	REACTION	TOTAL COMBINATIONS	NUMBER GIVING VISIBLE REACTION	NUMBER SHOWING NO NUCLEAR MIGRATION BEYOND DEMARKATION LINE	NUMBER TESTED
II	(Incompatible, A factors different) $AB \times aB$	Demarkation line formed	50	49	25	25
	$Ab \times ab$	Demarkation line formed	120	117	15	15

REACTION TYPE	GENOTYPES OF PAIRED MYCELIA	REACTION	TOTAL COMBINATIONS	NUMBER GIVING VISIBLE REACTION	NUMBER SHOWING NUCLEAR MIGRATION	NUMBER TESTED
III	(Incompatible, B factors different) $AB \times Ab$	Blotchy mycelium formed	120	119	19	20
	$AB \times ab$	Blotchy mycelium formed	50	49	10	10

REACTION TYPE	WITHIN MATING TYPES	REACTION	TOTAL COMBINATIONS	NO. GIVING REACTION	NO. TESTED	COMPLETELY UNILATERAL WITH COMPATIBLES	NUMBER TESTED
IV	$AB \times AB$	Vigorous mycelium	45	30	30	8	10
	$ab \times ab$	Vigorous mycelium	45	6	6	Not completed	..
	$Ab \times Ab$	Vigorous mycelium	66	6	6	5	5
	$aB \times aB$	Vigorous mycelium	10	6	6	Not completed	..

lateral behavior was found less frequently in the *Ab* × *aB* combinations. However, when it occurred the *Ab* mycelium always behaved as acceptor and the *aB* as donor.   Table 1 shows the frequency of these two types of diploidization.

Individual haploid mycelia of one mating type might display unilateral diploidization when paired with certain haploids of the compatible mating type but display regular diploidization with others.   At present, no definite pattern has been observed by means of which one can predict whether or not a given haploid belonging to one mating type will behave regularly or unilaterally when paired with a particular compatible haploid.   Individual haploids within one mating type do not appear to fall into definite groups in this respect.

When monosporous mycelia were stained, it was found that in the majority of them the hyphal tips were multinucleate, the young cells immediately behind the tip contained irregular numbers of nuclei and the older cells were uninucleate.   However, the hyphal tips of some *AB* and some *Ab* monosporous mycelia had a larger than average number of nuclei and most of the older cells of such mycelia contained two nuclei each. These two nuclei did not lie as close to one another as do the members of a regular dicaryon in true diploid mycelium.   Also, no evidence of conjugate division of the pairs of nuclei found in *AB* and *Ab* mycelia has yet been obtained.

In every example thus far investigated, the binucleate monosporous mycelium has shown unilateral diploidization when paired with certain compatible regularly uninucleate mycelia, and it has always behaved as an acceptor (see table 1).   Conversely, every *AB* or *Ab* mycelium which displayed unilateral diploidization with some compatible mycelia has, when stained, proved to be binucleate.

II.   Pairings of Incompatible Mycelia Having B or b Factors in Common (*AB* × *aB* and *Ab* × *ab*).—In these pairings a dense white line of demarkation developed in the region of contact between the two haploids. Mycelium taken from this line and examined microscopically consisted of wide, irregular cells, some of which bore incomplete or false clamp connections similar to those found by Brunswik[7] and others in combinations of incompatible mycelia.   Quintanilha[8] showed that mycelium bearing false clamp connections consists of some cells containing two nuclei and some containing only one.   The false clamp connection is formed during the conjugate division of the nuclei of the binucleate tip cells.

The mycelium on either side of the demarkation line remained uninucleate (if originally so) and unchanged in appearance.   When mycelium from either side of the line was paired with tester mycelia of each of the four mating types, it formed diploid mycelium only with the mating type with which it was originally compatible, indicating that nuclei had not mi-

grated beyond the demarkation line. The line reaction was seen in most $AB \times aB$ pairings and in most $Ab \times ab$, i.e., where the two mycelia had different A factors but possessed the B or b factor in common. The line reaction was not observed in other combinations (see table 1).

III. Pairings of Incompatible Mycelia Having A or a Factors in Common $(AB \times Ab$ *and* $aB \times ab)$.—In these pairings the two haploids grew together without a demarkation line. The mycelia resulting from such unions presented a very uneven growth throughout as to texture and color, and they grew more slowly than unpaired haploid mycelia. The term "blotchy" has been used to refer to this type of reaction.

When mycelium was taken from either side of a "blotchy" culture and paired with tester mycelia, it formed true diploid mycelium with the mating types with which both haploids had been compatible before pairing, i.e., the "blotchy" mycelium had become heterocaryotic. In some of the pairings of this "blotchy" mycelium with compatible monosporous mycelia, diploidization was unilateral, the "blotchy" mycelium acting as acceptor. More frequently, diploid mycelium grew out only from the region of contact between the "blotchy" and the monosporous mycelia. When stained, the "blotchy" mycelium was found to contain two nuclei in each cell, loosely associated in pairs, and similar in this respect to the unilateral AB and Ab mycelium described above. "Blotchy" reaction occurred in most pairings of $AB \times Ab$ and $aB \times ab$ and has not, as yet, been found elsewhere.

Binucleate monosporous AB or Ab mycelia (those which had exhibited unilateral diploidization when paired with a compatible uninucleate partner) also gave the "blotchy" reaction themselves when paired with tester mycelia which contained different B or b factors and similar A or a factors, but frequently did not cause the mycelium paired with them to become "blotchy." The uninucleate partner remained uninucleate and compatible only with the mating type with which it was originally compatible. From this it can be seen that the tendency for non-migration of nuclei exhibited when monosporous mycelia were paired with compatible partners was also exhibited in pairings with incompatible partners.

IV. Pairings within Mating Types $(AB \times AB,$ *etc.*).—When all possible combinations of AB haploids were made, a variety of interactions was observed. When two uninucleate AB mycelia were paired which had never shown unilateral diploidization with any ab mycelium, they gave rise to a tufted, irregularly binucleate mycelium. When this binucleate mycelium was paired with any ab haploid, it invariably exhibited unilateral diploidization and acted as the acceptor.

When a uninucleate regular AB mycelium was paired with a binucleate AB which was usually unilateral, the union produced a very coarse, vigorous mycelium which resembled diploid mycelium except for the lack of

true clamp connections and of color usually present in the true diploid of *Cyathus stercoreus*. Some cells of this mycelium were binucleate; others were uninucleate and bore false clamp connections. When this mixed *AB* mycelium was paired with *ab* mycelium, unilateral diploidization usually resulted and the *AB* mycelium acted as the acceptor. Similar results were obtained from other pairings within other mating types.

Discussion and Conclusions.—The results given above indicate that, in this series of monosporous mycelia derived from one fruit body of *Cyathus stercoreus*, the pair of factors *B–b* control nuclear migration. When two incompatible mycelia differing in these factors but containing the same *A* or *a* factor were paired, nuclear migration occurred and heterocaryotic "blotchy" mycelium developed. In combinations of incompatible mycelia which had the same *B* or *b* factors, extensive nuclear migration did not take place.

The pair of factors *A–a* would appear to be more closely involved in the establishment of the dicaryon. When two incompatible mycelia differing in these factors were paired, a mycelial line developed between the two. Mycelium taken from this line bore false clamp connections, indicating that nuclei of the two paired mycelia had entered a cell and formed a temporary dicaryon. No nuclear migration beyond this line took place.

However, other factors may also be involved in the compatibility reaction. Since some nuclei of the heterocaryotic mycelia *AB* × *Ab* and *aB* × *ab* were found in pairs, differences other than those of the *A–a* factors must, in these cases, be responsible for the attraction between two nuclei. Furthermore, since nuclear migration and the formation of false clamp connections occurred in pairings within a single mating type, these factors must segregate independently of mating type.

Whenever unilateral diploidization occurred, the acceptor mycelium was binucleate. This binucleate condition may have arisen in the spore or in the mycelium within the first three weeks after germination. It has been shown that it can be produced by pairing two different haploids of the same mating type or two haploids of different mating types in which the *B–b* pair of factors is present but only one of the *A–a* pair is present. In these cases, the two nuclei within a binucleate cell must be genetically different. This difference may be responsible, in some way, for the non-migration of nuclei.[9]

Each of the two commonly recognized pairs of compatibility factors apparently controls a different aspect of the diploidization process in *Cyathus stercoreus*. It is, however, also possible that certain functions are partially controlled by modifying factors located at different loci. Further analysis of these factors will be the object of later investigations.

Acknowledgment.—The writer wishes to express her gratitude to Dr. H. J. Brodie for his suggestions and criticism given during the course of this investigation.

[1] Kniep, H., *Verhandl. Physikal. Med. Ges. Wurzburg.*, **46**, 1–18 (1919).

[2] It should be noted that the use of the terms "compatible" and "incompatible" refers only to the interactions of mycelia determined by the mating type factors and that fusion of hyphae may take place between any two mycelia belonging to the same species, regardless of mating type. Compatible mycelia are those which when paired produce diploid mycelium bearing true clamp connections, each cell of the diploid mycelium containing a dicaryon.

[3] Buller, A. H. R., *Nature*, **126**, 686–689 (1930).

[4] Vandendries, R., and Brodie, H. J., *La Cellule*, **42**, 165–210 (1933).

[5] Dickson, H., *Ann. Bot.*, **49**, 181–204 (1935).

[6] Brodie, H. J., *Am. J. Bot.*, **35**, 312–320 (1948).

[7] Brunswik, H., *Z. Botan.*, **18**, 481–498 (1926).

[8] Quintanilha, A., *Bol. Soc. Brotereana*, **10**, 5–48 (1935).

[9] While this paper was in preparation, an abstract of work done by Papazian[10] was published which reported the results of a study of the interactions of mycelia of *Schizophyllum commune*. Papazian's findings are very similar to what has been reported in the preceding pages, in regard to the function of the *A* and *B* factors. As far as the writer can judge from the abstract, the two investigations concerning different species are mutually confirmatory.

[10] Papazian, H., *Am. J. Bot.*, **36** (suppl.), 813 (1949).

THE ACTION OF ALLELIC FORMS OF THE GENE A IN MAIZE. III. STUDIES ON THE OCCURRENCE OF ISOQUERCITRIN IN BROWN AND PURPLE PLANTS AND ITS LACK OF IDENTITY WITH THE BROWN PIGMENTS*

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The action of the A_1 gene has long been considered the standard example in maize of a gene-controlled, unit step in biosynthesis. This view rests on the work of Sando, *et al.*, who isolated the flavonol isoquercitrin (I) from brown husks of *aa B Pl* plants[1] and obtained the anthocyanin chrysanthemin (II) from purple husks of *A B Pl* plants.[2] From these findings it has been inferred generally that the *A* gene is concerned with the reduction at the 4-position either of isoquercitrin to chrysanthemin or of some precursor which these pigments have in common.

I   II
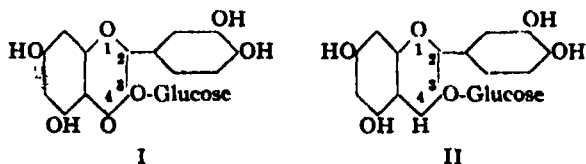
In spite of its appeal to simplicity this interpretation is subject to several reservations. (1) Although it is apparent from superficial observation alone

that the *A* gene determines the presence of purple pigment, it is not clear that isoquercitrin, known to be present in the brown plant, is absent or reduced in the purple plant as would be expected on the current hypothesis; there is no report of an attempt to isolate isoquercitrin from the purple plant.   (2) Pigmented tissues of the brown plant (*aa B Pl*) have yellowish brown cell walls whereas the cells of full-purple tissues (*A B Pl*) have purple vacuoles and colorless cell walls.   From a study[3] of these plants and others carrying different intermediate alleles of *A* it was concluded that the *A* alleles are concerned with some step affecting the relative quantities of purple and brown pigments.   Heretofore it has been assumed that the brown pigment is isoquercitrin but this identity is not established.   (3) Since the original isolation of chrysanthemin additional purple pigments have been recovered from *A B Pl* plants.[4]   Hence, even though it were established that isoquercitrin is affected by *A* action it is not valid to evaluate the action of *A* on the basis of the difference in structure between isoquercitrin and one of these purple pigments, chrysanthemin.

The present paper reports the lack of identity between the brown pigment of the *aa B Pl* plant and isoquercitrin and the occurrence of isoquercitrin in husks of purple maize.

Preliminary observations suggest that the brown pigment is not isoquercitrin.   (1) Aqueous extraction of the brown husks of *aa B Pl* plants yields a deep yellowish brown solution which becomes black on treatment with dilute ferric chloride solution; analogous extracts from green husks of *A b pl* plants give only a faint olive-green color with this test.   Brown pigments have been obtained free from the original extract of the brown plant and these give a black reaction with ferric chloride.   However, even relatively concentrated solutions of isoquercitrin produce only an olive-green color with this reagent.   (2) In dilute acid the brown pigment retains a strong yellowish brown color; on the other hand acid solutions of isoquercitrin are pale yellow to colorless.   (3) The separated pigment from the brown plant undergoes a reversible change from yellowish brown in acid solution to deep reddish brown in basic media; in contrast, the nearly colorless acid solution of isoquercitrin changes to bright yellow on addition of base.

A method has been devised which separates the brown and yellow pigments.   This technique, hereafter referred to as the "phase test," has much in common with the separation of phenols and certain organic acids by partition between ether and a water solution of a metal salt of a weak acid; this procedure, which has been modified somewhat since it was found necessary to use ethyl alcohol in place of ether, is as follows:

The aqueous extract of husks is treated with an equal volume of ethyl alcohol (95%) in a test-tube.   Successive portions of anhydrous potassium carbonate are added ac-

companied with slight agitation until the alcohol and water layers separate (under standard conditions 7 g. of carbonate per 10 ml. of extract are employed).

When this test is applied to the extract of brown *aa B Pl* plants the alcohol layer is bright yellow whereas the water layer is deep reddish brown. Since it might be argued that the pigment in the alcohol layer is the same as that in the water layer but present in lower concentration, it is significant that under the conditions of this test the distributions of the pigments to the respective layers are complete. That brown pigment is absent in the alcohol layer of this test is indicated by the behavior of several different brown pigments which have been isolated from extracts of *aa B Pl* plants according to procedures[5] which do not remove isoquercitrin; in independent phase tests each of these pigments is distributed completely to the water layer, the respective alcohol layers remaining colorless. Furthermore, the reactions of the yellow alcohol layer to color tests are negative for the brown pigments and are identical with those of isoquercitrin. Finally, the localization of isoquercitrin in the alcohol layer in these phase tests is supported by the behavior of a sample of crystalline isoquercitrin[6] which under identical test conditions distributes to the alcohol phase.

An attempt has been made to obtain spectrophotometric evidence of the presence of isoquercitrin in the alcohol layer of the phase test of plant extracts. From these efforts it is apparent that there are other substances present in this layer which absorb in the ultra-violet where isoquercitrin has its characteristic bands. Independent studies indicate that at least one of these substances is a flavonol distinct from isoquercitrin. In view of this difficulty separation by paper chromatography[7] was attempted. This technique has been employed recently in the separation of water-soluble plant pigments[8] and of mixtures of pure flavonols.[9] In adapting it to the isolation and subsequent spectrophotometric analysis of the pigments in the alcohol layer of the phase test the following procedure was employed:

Ten ml. of the original aqueous extract of husk tissue is diluted with an equal volume of 95% ethyl alcohol and the phases induced by addition of potassium carbonate. The alcohol layer is removed, centrifuged and neutralized with hydrochloric acid. It is then evaporated at room temperature and the pigment taken up in ethyl acetate leaving the neutralization salt behind. After evaporation of the ethyl acetate the pigment is taken up in a minimal quantity of a solution of water and 95% ethyl alcohol (1:3 volumes, respectively) which carries 1% HCl and is spotted 4 cm. from one end of a strip of Whatman No. 1 filter paper having the dimensions 5 cm. × 46 cm. In order to obtain an nitially narrow band spotting was effected with the edge of a microscope slide. The paper is suspended in a cylindrical hydrometer jar over a solution of butanol-acetic acid-water solvent (40:10:50 volume ratio). After 5 or 6 hrs. the strips are lowered to contact the solvent layer and movement of the solvent allowed to proceed from 24 to 48 hrs. (solvent travels from 30 to 40 cm.). Ordinarily two pigment solutions were partitioned on each strip, each jar accommodating three strips. To check the position of isoquercitrin on the test papers, a control paper spotted with the pure pigment was included in

each chamber. After air drying, the bands of pigment were developed with ammonia vapor, marked under ultra-violet light and cut from the strip. Pigments were eluted in 0.5% hydrochloric acid at room temperature and absorption curves obtained with a Beckman Model DU Quartz Spectrophotometer.

This procedure was followed in the analysis of brown and purple husks of individual maize plants. A comparison of the R_f values (ratio of distance traveled by the pigment on the paper strip to that traveled by the solvent) of pure isoquercitrin and the corresponding pigments from the tested plants (table 1) gives strong indication that both brown and purple

TABLE 1

THE R_f VALUES OF PURE ISOQUERCITRIN AND THE CORRESPONDING YELLOW PIGMENT
IN EXTRACTS OF BROWN ($aa\ B\ Pl$) AND PURPLE ($A\ B\ Pl$) PLANTS

Chromatogram series[a]	Source or plant no.	Phenotype	R_f
17	Isoquercitrin	...	0.66
17	49-923.1-1	Brown	0.68
17	49-923.1-2	Brown	0.69
17	49-923.1-3	Brown	0.68
17	49-923.1-4	Brown	0.68
18	Isoquercitrin	...	0.80
18	49-923.2-1	Purple	0.77
18	49-923.2-2	Purple	0.77
18	49-923.2-3	Purple	0.78
18	49-923.2-4	Purple	0.78
19	Isoquercitrin	...	0.80
19	49-227.1-1	Brown	0.81
19	49-227.2-1	Purple	0.80
19	49-227.2-2	Purple	0.80
23	Isoquercitrin	...	0.69
23	49-231.2-5	Brown	0.74
23	49-231.2-9	Brown	0.74
23	49-231.3-1	Purple	0.73
23	49-231.3-3	Purple	0.73
34	Isoquercitrin	...	0.60
34	45-545.2-3	Purple	0.64
34	45-545.2-6	Purple	0.63
34	45-545.2-8	Purple	0.62
34	45-545.2-10	Purple	0.63

[a] Each series comprises chromatograms developed in a single chamber and simultaneously.

plants contain isoquercitrin. Chromatograms of these plant extracts may show two additional bands of pigment which behave like isoquercitrin in that they give a yellow color with ammonia. One of these has an R_f of 0.75 that of isoquercitrin; the other occurs at or near the solvent front. Both are absent from control chromatograms of isoquercitrin.

The spectral absorption curve of pure isoquercitrin taken up in alcohol

and measured directly (curve 1, Fig. 1 A) has peaks at or close to 2575 A and 3625 A; minima at 2375 A and 2825 A; and a characteristic sloping plateau from 2950 A to 3150 A. Curve 2, figure 1 A, of isoquercitrin from a control chromatogram initially spotted with a concentrated solution of
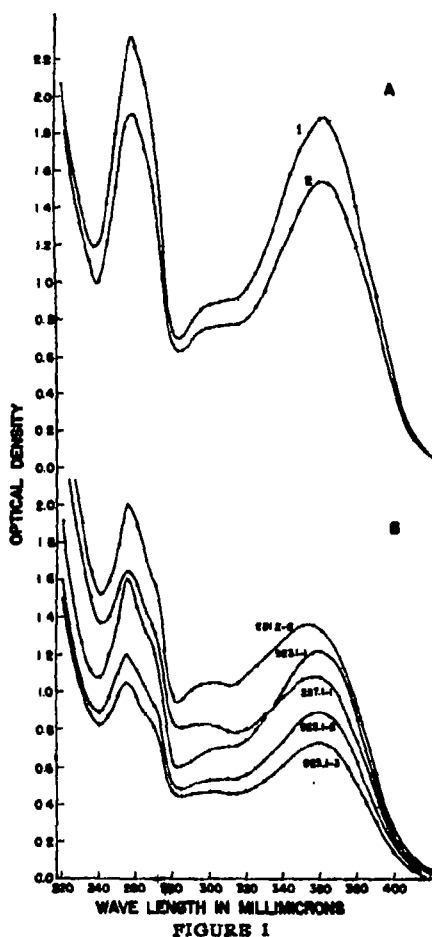
FIGURE 1

Spectral absorption curves of pigments following elution from paper chromatograms. (A) Curve 1, isoquercitrin measured directly; curve 2, isoquercitrin from control paper chromatogram. (B) The corresponding yellow pigments from chromatograms of preparations from the indicated brown (aa B Pl) plants. Pigments eluted and measured in 0.5% hydrochloric acid in 95% ethyl alcohol.
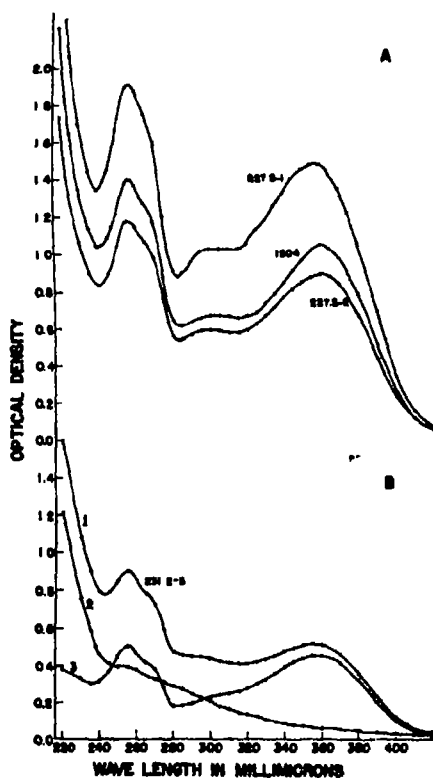
FIGURE 2

Spectral absorption curves of (A) the yellow pigments corresponding to isoquercitrin from chromatograms of preparations from the indicated purple (A B Pl) plants. (B) Curve 1, the yellow pigment corresponding to isoquercitrin from chromatogram of extract from the indicated brown plant; curve 2, the paper impurity eluted from a non-pigmented portion of the same chromatogram (see text for details); curve 3, plot of the differences in density values between curves 1 and 2. Pigments eluted and measured in 0.5% hydrochloric acid in 95% ethyl alcohol.

the pure pigment, is similar to curve 1, indicating that the absorption properties of the pigment are not modified by the paper partition method. Absorption curves of the yellow pigments from extracts of five brown (*aa B Pl*) plants are reproduced in figure 1 *B*. In each case the measurement was made on a solution obtained by elution of the yellow band having an R_f value similar to that of isoquercitrin on a corresponding control chromatogram. The presence of isoquercitrin in these plants is indicated by the close similarity of these curves to that of the pure pigment. However, there are several notable deviations: in the cases of the curves from the plants the absorption near 2200 A is relatively much greater, there is a tendency for both maxima to be displaced toward the shorter wave-lengths, for the minima to be displaced toward the longer wave-lengths and, instead of the gently sloping plateau in the 2950 A to 3150 A range, some of these curves exhibit a slight peak in this region. These deviations are explained if it is assumed that the measured solutions carried, in addition to isoquercitrin, a substance whose absorption is great in the 2200 A region and diminishes steadily on approaching the visible range. That such a substance is present on the paper chromatograms is indicated by the curves in figure 2 *B*. Here the uppermost curve comes from an elution of that portion of a paper chromatogram carrying the isoquercitrin band from the indicated brown plant; in general character it resembles that of pure isoquercitrin but deviates from it in the same direction as, and to an even greater extent than the curves in figure 1 *B*. A portion of this same paper strip taken from 3 cm. below the isoquercitrin band in a region which gave no fluorescence with ultra-violet light was removed. This blank piece was equal in size to that which gave curve 1, the two being handled simultaneously and under identical conditions. The solution obtained by extraction of this non-pigmented portion of the paper gave curve 2, figure 2 *B*. On the assumption that the paper impurity is present throughout the chromatogram and was present in the isoquercitrin band also, it is valid to plot a corrected curve for isoquercitrin from the differences between the density values of curves 1 and 2. This adjusted curve (Fig. 2 *B*, curve 3) is similar to that expected for isoquercitrin.

As noted in table 1, on each chromatogram of the purple (*A B Pl*) plants there is a yellow band corresponding to isoquercitrin. It is significant that in all cases these bands are much less intense than those from the brown plants suggesting that less of this pigment occurs in purple plants. This is borne out by the absorption curves obtained from these bands. In all tested cases (six bands were analyzed) extremely low density values were obtained and the curves resembled that of the paper impurity (Fig. 2 *B*) giving only slight indication of the presence of isoquercitrin. By an alternate procedure larger amounts of isoquercitrin were obtained for initial spotting on the papers:

Fifty ml. of the original aqueous extracts of purple husks were extracted exhaustively with ether. After evaporation of the ether portions the residue was taken up in the aqueous-alcoholic spotting solvent and the chromatograms developed as described previously.

The absorption curves of the yellow bands from three purple plants obtained following this procedure are reproduced in figure 2 *A*. Clearly, these indicate the presence of isoquercitrin in the purple plant.

The solutions which gave the spectral absorption curves typical for isoquercitrin in figure 1 *B* were prepared from the alcohol layer of the phase test of brown plant extracts. Since the brown pigments are excluded from this layer it may be concluded that these are distinct from isoquercitrin. This distinction makes it necessary to take account of the brown pigments in any scheme representing *A* action, since it is known that the extent of their occurrence is influenced by the *A* gene present.

While the evidence presented here indicates that isoquercitrin is present in both brown (*aa B Pl*) and purple (*A B Pl*) plants it is not reasonable to conclude that the *A* alleles do not affect this pigment, since there is strong indication that less of it is present in plants of the latter type. Nevertheless, since the *A* gene is now known to affect the synthesis of a number of related chemical substances (viz., the brown pigments, and the purple pigments in addition to chrysanthemin) there is little basis for the generally accepted view that the action of this gene is concerned solely if at all with the oxidation-reduction step which makes the difference between isoquercitrin and chrysanthemin.

* A portion of the work reported here was conducted at the University of Missouri.

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3 Laughnan, J. R., *Genetics*, 33, 488–517 (1948).

4 Laughnan, J. R., *Ibid.* (abstract), 31, 222 (1946).

5 The investigations of the brown pigments and their significance from the standpoint of action of the *A* gene will be reported elsewhere.

6 This sample traces to the original pigment isolated from brown maize by Sando *et al*. Its receipt from Prof. M. M. Rhoades, who obtained it from the late Prof. R. ... Emerson, is gratefully acknowledged.

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DETERMINATION OF THE SPECTRUM OF THE FLOW OF BROWNIAN MOTION

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1. Brownian Motion.—The Brownian motion of N. Wiener[1] can be defined as follows: Let $(\Omega, \mathfrak{E}, Pr)$ be a *probability space*, i.e., $\Omega = \{\omega\}$ is a set of elements ω, $\mathfrak{E} = \{E\}$ is a Borel field of subsets E of Ω, and $Pr(E)$ is a countably additive measure defined on $\mathfrak{E}$ satisfying the condition $Pr(\Omega) = 1$. Let further $S = \{s \mid -\infty < s < \infty\}$ be a one-dimensional Euclidean space, and let $\mathfrak{J} = \{I\}$ be the family of all finite intervals $I = (a, b) = \{s \mid a < s \leq b\}$. We denote by $|I|$ the length $b - a$ of the interval $I = (a, b)$, and $I = I_1 + I_2$ means that I is a union of two intervals I_1 and I_2 disjoint from each other.

A real-valued function $x(I, \omega)$ defined for $I \in \mathfrak{J}$ and $\omega \in \Omega$ is a *Brownian motion* if the following conditions (A), (B), (C) are satisfied:

(A) For any fixed $I \in \mathfrak{J}$, $x(I, \omega)$ is a measurable function of ω on Ω and has a Gaussian distribution with mean value 0 and variance $\sigma = |I|$, i.e.,

$$E_{I, \alpha, \beta} \equiv \{\omega \mid \alpha < x(I, \omega) < \beta\} \in \mathfrak{E} \tag{1}$$

$$Pr(E_{I, \alpha, \beta}) = \frac{1}{\sqrt{2\pi\sigma}} \int_\alpha^\beta e^{-u^2/2\sigma} \, du \tag{2}$$

for any $I \in \mathfrak{J}$ and for any real numbers α, β with $\alpha < \beta$.

(B).   For any finite number of mutually disjoint intervals $I_k \in \mathfrak{J}$, $k = 1, \ldots, n$, the corresponding functions $x(I_k, \omega)$, $k = 1, \ldots, n$, are independent in the sense of probability, i.e.,

$$Pr(\bigcap_{k=1}^n E_{I_k, \alpha_k, \beta_k}) = \Pi_{k=1}^n Pr(E_{I_k, \alpha_k, \beta_k}) \tag{3}$$

for any real numbers α_k, β_k with $\alpha_k < \beta_k$, $k = 1, \ldots, n$.

(C) $x(I, \omega)$ is additive in I, i.e.,

$$x(I, \omega) = x(I_1, \omega) + x(I_2, \omega) \tag{4}$$

almost everywhere on Ω if $I = I_1 + I_2$.

A Brownian motion $x(I, \omega)$ is *fundamental* if the following condition is satisfied:

(D) The Borel field $\mathfrak{E}'$ generated by the family $\{E_{I, \alpha, \beta} \mid I \in \mathfrak{J}, -\infty < \alpha < \beta < \infty\}$ is dense in $\mathfrak{E}$, i.e., for any $E \in \mathfrak{E}$ there exists an $E' \in \mathfrak{E}'$ such that $Pr(E \cup E' - E \cap E') = 0$.

Let $L^2(\Omega) = L^2(\Omega, \mathfrak{E}, Pr)$ be the L^2-space of all real-valued $\mathfrak{E}$-measurable functions $f(\omega)$ defined on Ω with

$$\|f\| = (\int_\Omega |f(\omega)|^2 Pr(d\omega))^{1/2} < \infty \qquad (5)$$

as its norm. It is easy to see that $x(I, \omega) \in L^2(\Omega)$ for any $I \in \mathfrak{I}$. Further, for any finite number of intervals $I_k \in \mathfrak{I}$, $k = 1, \ldots, n$, the product function $\Pi_{k=1}^n x(I_k, \omega)$ belongs to $L^2(\Omega)$, too. Consequently, the ring $\mathfrak{R}$ generated by the functions $x(I, \omega)$, $I \in \mathfrak{I}$, is a subset of $L^2(\Omega)$. It is then easy to see that (D) is equivalent to

  (D') $\mathfrak{R}$ is a dense subset of $L^2(\Omega)$, i.e., for any $f(\omega) \in L^2(\Omega)$ and for any $\epsilon > 0$ there exists a function $x(\omega)$ of the form:

$$x(\omega) = \sum_{p=1}^q a_p \Pi_{k=1}^{n_p} x(I_{p, k}, \omega) \qquad (6)$$

such that $\|f - x\| < \epsilon$, where $I_{p, k} \in \mathfrak{I}$, $k = 1, \ldots, n_p$; $p = 1, \ldots, q$; and a_p is a real number, $p = 1, \ldots, q$.

  2. *Flow of a Brownian Motion.*—Let us assume that our probability space $(\Omega, \mathfrak{E}, Pr)$ is the Lebesgue measure space (i.e., Ω is the set of all real numbers ω, $0 \leq \omega \leq 1$; $\mathfrak{E}$ is the Borel field of all Lebesgue measurable subsets E of Ω, and $Pr(E)$ is the ordinary Lebesgue measure of E with the normalization $Pr(\Omega) = 1$), or any other measure space spatially isomorphic with it.

  LEMMA. *Let $x(I, \omega)$ be a fundamental Brownian motion. Then there exists a flow $\{\varphi_t\}$ defined on $(\Omega, \mathfrak{E}, Pr)$ (i.e., a one-parameter group of measure-preserving transformations $\omega' = \varphi_t(\omega)$ defined on $(\Omega, \mathfrak{E}, Pr)$ satisfying $\varphi_s(\varphi_t(\omega)) = \varphi_{s+t}(\omega)$ almost everywhere on Ω for any s and t) such that*

$$x(I + t, \omega) = x(I, \varphi_t(\omega)) \qquad (7)$$

almost everywhere on Ω for any $I \in \mathfrak{I}$ and for any t $(-\infty < t < \infty)$, where $I + t = (a + t, b + t)$ is the interval obtained from $I = (a, b)$ by the translation $s \to s + t$.

  This flow $\{\varphi_t\}$ is called *the flow of a Brownian motion $x(I, \omega)$.* The purpose of this note is to determine the spectral type of the corresponding one-parameter group of unitary transformations $\{U_t\}$ defined on $L^2(\Omega)$ by

$$U_t f(\omega) = f(\varphi_t(\omega)). \qquad (8)$$

  It was proved by N. Wiener[2] and E. Hopf[2] that $\{U_t\}$ is strongly mixing, i.e., that

$$\lim_{t \to \infty} Pr(\varphi_t(E) \cap F) = Pr(E) \cdot Pr(F) \qquad (9)$$

for any E, $F \in \mathfrak{E}$, or equivalently that

$$\lim_{t \to \infty} (U_t f, g) = (f, 1) \cdot (1, g) \qquad (10)$$

for any f, $g \in L^2(\Omega)$, where (f, g) denotes the inner product of f and g in $L^2(\Omega)$. Further, it was proved by H. Anzai[1] that $\{U_t\}$ has an absolutely

continuous spectrum, i.e., that, for any $f \, \epsilon \, L^2(\Omega)$, there exists an absolutely continuous monotone non-decreasing function $\sigma(\lambda)$ defined for $-\infty < \lambda < \infty$ such that

$$(U_t f, f) = \int_{-\infty}^{\infty} e^{it\lambda} \, d\sigma(\lambda) \tag{11}$$

for any real number t, $-\infty < t < \infty$.

3. Polynomial Chaos and Spectral Decomposition of the Flow of a Brownian Motion.—In order to state our result, let us first denote by $\mathfrak{P}_n$ the set of all $x(\omega) \, \epsilon \, L^2(\Omega)$ of the form (6) in which $n_p \leqq n$, $p = 1, \ldots, q$. $\mathfrak{P}_n$ is called the *polynomial chaos* of degree n. It is easy to see that each $\mathfrak{P}_n$ is a linear subspace of $L^2(\Omega)$ which is invariant under $\{U_t\}$, and that $\mathfrak{P}_{n-1} \subset \mathfrak{P}_n$, $n = 1, 2, \ldots$. Further, the union $\bigcup_{n=1}^{\infty} \mathfrak{P}_n$ of all $\mathfrak{P}_n$ is clearly equal to $\mathfrak{R}$ and hence is dense in $L^2(\Omega)$ by assumption. Let $\mathfrak{M}_n$ be the ortho-complement of $\bar{\mathfrak{P}}_{n-1}$ in $\bar{\mathfrak{P}}_n$, where $\bar{\mathfrak{P}}_{n-1}$ and $\bar{\mathfrak{P}}_n$ denote the closures of $\mathfrak{P}_{n-1}$ and $\mathfrak{P}_n$, respectively. Then it is easy to see that each $\mathfrak{M}_n$ is a closed linear subspace of $L^2(\Omega)$, invariant under $\{U_t\}$; that $\mathfrak{M}_n$, $n = 0, 1, 2, \ldots$, are mutually orthogonal and span the whole space $L^2(\Omega)$.

On the other hand, let $S^n = S \times \ldots \times S$ (n times) be the n-dimensional Euclidean space, and let $L^2(S^n)$ be the L^2-space of all real-valued Lebesgue measurable functions $f(s_1, \ldots, s_n)$ defined on S^n with

$$\|f\| = \left(\int_{-\infty}^{\infty} \ldots \int_{-\infty}^{\infty} |f(s_1, \ldots, s_n)|^2 ds_1 \ldots ds_n \right)^{1/2} < \infty \tag{12}$$

as its norm, where the integral is the ordinary n-dimensional Lebesgue integral. It is clear that

$$V_t^{(n)} f(s_1, \ldots, s_n) = f(s_1 + t, \ldots, s_n + t) \tag{13}$$

defines a unitary transformation $V_t^{(n)}$ of $L^2(S^n)$ onto itself. Let further $L_0^2(S^n)$ be a subspace of $L^2(S^n)$ consisting of all $f(s_1, \ldots, s_n) \, \epsilon \, L^2(S^n)$ which are symmetric functions of $s_1, \ldots, s_n$. $L_0^2(S^n)$ is clearly a closed linear subspace of $L^2(S^n)$ and is invariant under $\{V_t^{(n)}\}$.

Then our main result can be stated as follows:

THEOREM. *$L^2(\Omega)$ can be decomposed into a direct sum of a countable infinite number of mutually orthogonal closed linear subspaces $\mathfrak{M}_n$, $n = 0, 1, 2, \ldots$, in such a way that (i) each $\mathfrak{M}_n$ is invariant under $\{U_t\}$ and (ii) $\{U_t\}$ on $\mathfrak{M}_n$ is spectrally isomorphic with $\{V_t^{(n)}\}$ on $L_0^2(S^n)$.*

The last statement of this theorem means that it is possible to find a linear isometric mapping W_n of $\mathfrak{M}_n$ onto $L_0^2(S^n)$ which satisfies $W_n^{-1} V_t^{(n)} W_n = U_t$ for any real number t, $-\infty < t < \infty$.

4. Hermite Polynomials.—The proof of our theorem can be carried out by using the Hermite polynomials $H_n(u, \sigma)$ with a parameter $\sigma > 0$, which are defined by the following formula:

$$H_n(u, \sigma) = (-1)^n (n!)^{-1} \sigma^n \cdot e^{u^2/2\sigma} \cdot \frac{d^n}{du^n} (e^{-u^2/2\sigma}) \tag{14}$$

$-\infty < u < \infty$, $\sigma > 0$, $n = 0, 1, 2, \ldots$. $H_n(u, \sigma)$ is clearly a polynomial of degree n in u and it is easy to see that the coefficient of u^n is $(n!)^{-1}$. It is well known that, for any fixed $\sigma > 0$, $H_n(u, \sigma)$, $n = 0, 1, 2, \ldots$, form an orthogonal system in the infinite interval $-\infty < u < \infty$ with respect to a weight function exp $(-u^2/2\sigma)$, or more precisely

$$I_{m, n} = \frac{1}{\sqrt{2\pi\sigma}} \int_{-\infty}^{\infty} H_m(u, \sigma) H_n(u, \sigma) e^{-u^2/2\sigma} \, du$$

$$= \begin{cases} 0, & m \neq n, \quad m \geq 0, \quad n \geq 0, \qquad (15) \\ \dfrac{\sigma^n}{n!}, & m = n \geq 0. \end{cases}$$

Further, it is easy to see that $H_n(u, \sigma)$ satisfies the following functional relation:

$$H_n(u + v, \sigma + \tau) = \sum_{k=0}^{n} H_k(u, \sigma) \cdot H_{n-k}(v, \tau) \qquad (16)$$

for $-\infty < u, v < \infty$ and $\sigma, \tau > 0$.

5. *Homogeneous Polynomial Chaos.*—Let us now consider the set $\mathfrak{Q}_n$ of all $x(\omega) \in L^2(\Omega)$ of the form:

$$x(\omega) = \sum_{(n_1, \ldots, n_p)} a_{n_1, \ldots, n_p} \Pi_{k=1}^{p} H_{n_k}(x(I_k, \omega), \sigma_k), \qquad (17)$$

where $\{I_k \mid k = 1, \ldots, p\}$ is any finite system of mutually disjoint intervals from $\mathfrak{J}$; $\sigma_k = |I_k|$, $k = 1, \ldots, p$; $a_{n_1, \ldots, n_p}$ is a real number for each $(n_1, \ldots, n_p)$, and $\sum_{(n_1, \ldots, n_p)}$ denotes the sum for all p-systems of non-negative integers $(n_1, \ldots, n_p)$ such that $n_1 + \ldots + n_p = n$. $\mathfrak{Q}_n$ is called the *homogeneous polynomial chaos* of homogeneous degree n.

It is not difficult to see that each $\mathfrak{Q}_n$ is a linear subspace of $L^2(\Omega)$ which is invariant under $\{U_t\}$, that any two $\mathfrak{Q}_m$, $\mathfrak{Q}_n (m \neq n)$ are orthogonal to each other and that $\mathfrak{P}_n = \mathfrak{Q}_0 + \mathfrak{Q}_1 + \ldots + \mathfrak{Q}_n$, for $n = 0, 1, 2, \ldots$. From this follows easily that $\bar{\mathfrak{P}}_n = \bar{\mathfrak{Q}}_0 + \bar{\mathfrak{Q}}_1 + \ldots + \bar{\mathfrak{Q}}_n$ and $\mathfrak{M}_n = \bar{\mathfrak{P}}_n$, $n = 0, 1, 2, \ldots$, where we denote by $\bar{\mathfrak{P}}_n$, $\bar{\mathfrak{Q}}_n$ the closures of $\mathfrak{P}_n$, $\mathfrak{Q}_n$, respectively.

Let us now put $f = W_n x$ if $x(\omega) \in \mathfrak{Q}_n$ is of the form (17) and if $f(s_1, \ldots, s_n) \in L_0^2(S^n)$ is of the form:

$$f(s_1, \ldots, s_n) = (n!)^{-1/2} \sum_{(n_1, \ldots, n_p)} a_{n_1, \ldots, n_p} \sum_{\{J\}} \Pi_{i=1}^{n} \chi_{J_i}(s_i), \qquad (18)$$

where $\chi_J(s)$ is the characteristic function of a set J; $(J_1, \ldots, J_n)$ is a permutation of $(I_1, \ldots, I_1 \ (n_1 \text{ times}), I_2, \ldots, I_2 \ (n_2 \text{ times}), \ldots, I_p, \ldots, I_p \ (n_p \text{ times}))$; $\sum_{\{J\}}$ means the sum for all possible different permutations of $(J_1, \ldots, J_n)$ (note that there are $n!/n_1! \ldots n_p!$ different permutations); $a_{n_1, \ldots, n_p}$ is a real number for each $(n_1, \ldots, n_p)$ and $\sum_{(n_1, \ldots, n_p)}$ denotes the same thing as in (17). In other words, (18) means that $f(s_1, \ldots, s_n) = (n!)^{-1/2} a_{n_1, \ldots, n_p}$ if all s_i, $i = 1, \ldots, n$, belong to $\bigcup_{k=1}^{p} I_k$ and

if the number of elements s_i which belong to I_k is n_k for $k = 1, \ldots, p$. Otherwise (i.e., if at least one element s_i, $1 \leq i \leq n$, does not belong to $\bigcup_{k=1}^{p} I_k$), $f(s_1, \ldots, s_n) = 0$.

It is then easy to see that W_n is a linear transformation of $\mathfrak{Q}_n$ onto a dense linear subspace of $L_0^2(S^n)$ which we shall denote by $\mathfrak{Q}_n'$. It is also easy to see that

$$\|x\|^2 = \sum_{(n_1, \ldots, n_p)} |a_{n_1, \ldots, n_p}|^2 \, \Pi_{k=1}^{p} \frac{\sigma_k^{n_k}}{n_k!} \qquad (19)$$

if $x(\omega) \in \mathfrak{Q}_n$ is of the form (17) and that

$$\|f\|^2 = (n!)^{-1} \sum_{(n_1, \ldots, n_p)} |a_{n_1, \ldots, n_p}|^2 \frac{n!}{n_1! \ldots n_p!} \, \Pi_{k=1}^{p} \sigma_k^{n_k} \qquad (20)$$

if $f(s_1, \ldots, s_n) \in \mathfrak{Q}_n'$ is of the form (18). This shows that W_n is an isometric transformation of $\mathfrak{Q}_n$ onto $\mathfrak{Q}_n'$. Further it is clear from the definition of U_t, $V_t^{(n)}$ and W_n that $U_t = W_n^{-1} V_t^{(n)} W_n$ on $\mathfrak{Q}_n$ for any t, $-\infty < t < \infty$. Since $\mathfrak{Q}_n$ and $\mathfrak{Q}_n'$ are dense in $\mathfrak{M}_n$ and $L_0^2(S^n)$, respectively, it is possible to extend W_n to a linear isometric transformation (which we shall again denote by W_n) of $\mathfrak{M}_n$ onto $L^2(S^n)$ which satisfies $U_t = W_n^{-1} V_t^{(n)} W_n$ on $\mathfrak{M}_n$ for any t, $-\infty < t < \infty$. This completes the proof of the Theorem.

It is easy to see that the results of Wiener, Hopf and Anzai stated in § 2 follow immediately from our Theorem. The decomposition of $L^2(\Omega)$ into a direct sum of mutually orthogonal invariant linear subspaces $\mathfrak{M}_n$, $n = 0, 1, 2, \ldots$, on each of which our $\{U_t\}$ behaves very nicely, makes the situation very simple and easy to handle. The notion of polynomial chaos $\mathfrak{P}_n$ and the discovery of its usefulness in our problem of spectral analysis are due to N. Wiener.[3] Wiener's original argument was more complicated since he discussed only $\mathfrak{P}_n$ and not $\mathfrak{M}_n$ nor $\mathfrak{Q}_n$. The idea of taking the ortho-complement $\mathfrak{M}_n$ of $\mathfrak{P}_{n-1}$ in $\mathfrak{P}_n$ is due to J. von Neumann (oral communication). The introduction of Hermite polynomials $H_n(u, \sigma)$ with a parameter $\sigma > 0$ satisfying the functional relation (16), and the definition (17) of homogeneous polynomial chaos $\mathfrak{Q}_n$ in terms of these Hermite polynomials $H_n(u, \sigma)$ made it easy to set up an isomorphism between $\mathfrak{M}_n$ and $L_0^2(S^n)$ which carries $\{U_t\}$ over into $\{V_t^{(n)}\}$. Similar results were also obtained recently by K. Ito in a different way by using the method of stochastic integral.

The detail of the proof and the discussion of allied problems are left to a subsequent paper where a more general class of Brownian motions will be treated.

[1] Anzai, H., "A Remark on Spectral Measures of the Flow of Brownian Motion," *Osaka Math. J.*, 1 95–97 (1949).

[2] Hopf ,E., *Ergodentheorie*, 1937. §14.

[3] Wiener, N., "The Homogeneous Chaos," *Am. J. Math.*, 60 897–936 (1939).

THE CRYSTALLOGRAPHIC SYMMETRIES DETERMINABLE BY X-RAY DIFFRACTION

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The problem of how to determine the symmetry of a given crystal has been an important one from the early days of the science of crystallography. Symmetry requires the various properties of a crystal to be equivalent in symmetrically equivalent directions. Accordingly, the general nature of a test for the presence or absence of a particular kind of symmetry is to subject the possibly symmetrical directions of a crystal to some physical or chemical action and then ascertain whether the crystal's response is equivalent in these directions or not.

Unfortunately, some tests inherently involve certain symmetry themselves and this prevents one from judging whether the symmetry resulting from such a test derives from the crystal or from the test. This kind of dilemma occurs in testing the symmetry of a crystal by its diffraction effects. When a crystal diffracts x-rays whose wave-length is not in the immediate neighborhood of an absorption edge of one of the chemical elements in the crystal, the diffraction effects are inherently centrosymmetrical. This was pointed out by Friedel[1] as early as 1913, only a year after the discovery of the diffraction of x-rays by crystals made by von Laue and his students.[2, 3, 4] The cause of this lies in the impossibility of experimentally observing the phases of the x-ray diffraction spectra. Thus, if a set of planes hkl "reflects" x-rays with amplitude $|F_{hkl}|$ and phase φ, the set of planes $\bar{h}\bar{k}\bar{l}$ "reflects" with identical amplitude $|F_{hkl}|$, but with phase $-\varphi$. Since the amplitudes (in the form of intensities, or F^2's) but not the phases of the reflections can be experimentally observed, diffraction effects are inherently centrosymmetrical, and, even though a crystal does not have a center of symmetry, its diffraction effects do display a center. Therefore, although there are 32 symmetry combinations possible in crystals, only 11 symmetry combinations can be distinguished by the symmetry of their diffraction effects. None of these 11 sets uniquely characterizes any of the 32 crystal classes (i.e., the 32 combinations of symmetry possible for crystals).

One might suppose that this feature provides an ultimate limitation on the detection of symmetry by x-ray diffraction. But in 1919 Niggli[5] showed that the space group symmetry elements which involve translation components could be identified by the characteristic missing spectra which they caused to occur in the diffraction effects. Later the writer[6, 7] showed that by combining the information from Friedel's law and Niggli's "ex-

tinctions" (missing spectra), a total of 120 "diffraction groups" could be distinguished. The total number of space groups, or kinds of internal symmetry which are possible in crystals, is 230. Most of the diffraction groups include several space groups, namely, those from the several centrosymmetrical crystal classes which have the same Niggli extinctions. But 59 of these diffraction groups embrace only a single space group (counting enantiomorphous pairs as one space group) and consequently 59 out of the 219 space groups (excluding enantiomorphous duplicates) can be distinguished by a mere qualitative inspection of the diffraction data.

At the 1946 meeting of the American Society for X-Ray and Electron Diffraction, the writer announced[8] that all space groups except those pairs differing solely by an inversion could be distinguished by diffraction means provided that the spectra are considered on a quantitative basis. In the light of this discovery, Niggli's extinctions, which amounted to *intensity* = 0 for certain special spectra, were seen to be merely a qualitatively obvious special case of a much more general control of spectral intensity by the crystal symmetry. It thus became clear that the key to crystal symmetry was contained in the diffraction spectra of the crystal.

In the same paper[8] the writer showed that the key to the structure of the crystal also lay fallow in the spectra, and one device for finding the structure, namely, the *implication* method, was suggested. This was an astonishing result which was received with incredulity by nearly everyone, because it was a well-established belief[9] that, since the phases of the diffraction cannot be observed, there is not necessarily a solution for a crystal structure from diffraction data alone.

Some more recent developments make it possible to treat the determinability of crystal symmetry by x-ray diffraction data on a more straightforward basis. The electron density of a crystal at a selection of points xyz is related to the complex amplitudes, F_{hkl}, of the diffraction spectra hkl by

$$\rho(xyz) = \sum_{h}\sum_{k}\sum_{l}^{\infty} F_{hkl} e^{2\pi i(hx + ky + lz)}. \tag{1}$$

Patterson[10, 11] showed that another function, not involving the complex F's, but rather their observable squares, bears a simple relation to (1). Patterson's function,

$$A(uvw) = \sum_{h}\sum_{k}\sum_{l}^{\infty} F^2_{hkl} e^{2\pi i(hu + kv + lw)}, \tag{2}$$

has maxima where vectors with components u, v, w span maxima in (1). Thus, if maxima occur at $x_1y_1z_1$ and $x_2y_2z_2$ in (1), then maxima occur in (2) at uvw, where

$$\left.\begin{array}{l} u = x_2 - x_1 \\ v = y_2 - y_1 \\ w = z_2 - z_1 \end{array}\right\}. \tag{3}$$

Of course, maxima occur in (1) where the electron density is high, i.e., at atom locations.

The relation involved between (1) and (2) can be rendered more evident if points are substituted for atoms in the crystal structure.[11] Then (1) is transformed into a collection of points called the *fundamental set*, and (2) is transformed into another collection of points called the *vector set*. To derive the vector set from the fundamental set, the vectors between all pairs of points in the fundamental set are drawn, then the vectors are reassembled at a common origin. The collection of points at the ends of the reassembled vectors constitutes the vector set.

It is an easy matter to find the vector set for any given fundamental set. The writer has recently shown[11] that it is also possible to find the fundamental set for any vector set. But the vector set is a representation of (2), simplified so that the maxima become points,[11] and the fundamental set is a representative of (1), similarly simplified. This implies that if (2) can be solved, (1) can be derived from it. The interesting consequence of this is that, although (1) cannot be computed from diffraction data because of the unobservability of the phase component of the complex F's, (2) involves F^2's and therefore does not require a knowledge of these phase components. Therefore, in principle, crystal structures are solvable from diffraction data alone.

An immediate consequence of this conclusion is that all crystal symmetries are determinable from diffraction data, for the symmetry of a crystal is an incidental aspect of its structure. Furthermore, since the symmetry of the fundamental set and the crystal are the same, and since the fundamental set is derivable from the vector set, it follows that the key to the symmetry of the crystal is in its vector set.

Actually, vector sets display fewer possible symmetries than the fundamental sets, since the former must all be centrosymmetrical. The writer has shown another way of looking at this.[11] When the vectors between points in the fundamental set are transferred to the origin of the vector set, the translation components of the symmetry elements relating the vectors are lost to the symmetry of the vector set, although other components are retained. In this way it can be shown that, although the symmetries of the fundamental sets comprise the 230 space groups, there are only 24 space group symmetries possible in vector sets. But in this degeneration of actual symmetry, the key to the symmetry of the original fundamental set is not lost. The translation components of the vectors between all atoms in the fundamental set which are symmetrically equivalent,

when transferred to the vector set, cause a concentration of points at the ends of these vectors at loci characteristic of the lost translation. The principal key to crystallographic symmetry is these concentrations in vector space. These are discovered for any crystal by computing the full three-dimensional Patterson synthesis (2). The unaided computation of this synthesis is so laborious that it has very rarely been carried out to date. But with the recent development of the Pepinsky Fourier synthesizer,[14] this full synthesis has now become quite practical.

The writer has tabulated the symmetrical concentrations for the 230 space groups. The tabulation shows that all space groups are uniquely

TABLE 1

PAIRS OF SPACE GROUPS NOT DISTINGUISHABLE BY LOCATIONS OF SYMMETRICAL IMAGES

THE ENANTIOMORPHOUS PAIRS

$\begin{cases} C3_1 \\ C3_2 \end{cases}$	$\begin{cases} P1 \\ P\bar{1} \end{cases}$	$\begin{cases} C\bar{6} \\ C6/m \end{cases}$	$\begin{cases} I222 \\ I2_12_12_1 \end{cases}$
$\begin{cases} C3_121 \\ C3_221 \end{cases}$	$\begin{cases} C3 \\ C\bar{3} \end{cases}$		$\begin{cases} I23 \\ I2_13 \end{cases}$
$\begin{cases} C3_112 \\ C3_212 \end{cases}$	$\begin{cases} R3 \\ R\bar{3} \end{cases}$		
$\begin{cases} P4_1 \\ P4_3 \end{cases}$	$\begin{cases} P4 \\ P\bar{4} \end{cases}$		
$\begin{cases} P4_12 \\ P4_32 \end{cases}$	$\begin{cases} I4 \\ I\bar{4} \end{cases}$		
$\begin{cases} P4_12_1 \\ P4_32_1 \end{cases}$			
$\begin{cases} C6_1 \\ C6_5 \end{cases}$			
$\begin{cases} C6_2 \\ C6_4 \end{cases}$			
$\begin{cases} C6_122 \\ C6_522 \end{cases}$			
$\begin{cases} C6_222 \\ C6_422 \end{cases}$			
$\begin{cases} P4_132 \\ P4_332 \end{cases}$			

characterized by these concentrations except for the pairs shown in table 1. There are four kinds of categories where the concentrations determine pairs of space groups instead of a single space group. Each category is listed as a column in table 1. Column one contains the 11 pairs of enantiomorphous space groups. Column two contains 4 pairs of space groups, each pair containing a cyclical group of the first sort and the corresponding cyclical group of the second sort. Columns three and four contain three more pairs which cannot be placed in either of the other categories.

Now, all space groups except the 19 pairs shown in table 1 can be distinguished merely by the locations of the concentrations. But by taking into account some obvious aspects of the *patterns* in the concentrations, all space groups except the 11 enantiomorphous pairs in the first column of table 1 can be distinguished. Thus, in the pairs $P4$, $P\bar{4}$ and $I4$, $I\bar{4}$ the first member of each pair contains satellites[8] while the second does not. (A satellite is merely a duplication of the pattern motif enlarged and rotate 45° in this case.) The members of the pairs are therefore easily distinguished by the qualitative appearance of the patterns in the concentration. In the first member of the pairs $I222$, $I2_12_12_1$ and $I23$, $I2_13$, the patterns in the three orthogonal (axial) planar concentrations are simple projections of one another. For the second member of the pairs, the corresponding patterns are also projections of one another, but with origins shifted $\frac{1}{4}\frac{1}{4}0$ etc. The members of these pairs can therefore also be distinguished by the qualitative appearance of the patterns in the concentrations. It remains to distinguish between members of the three pairs $P1$, $P\bar{1}$; $R3$, $R\bar{3}$; and $C3$, $C\bar{3}$. These can be distinguished if it is possible to distinguish points of weight 1 and weight 2, since the first and second members of these pairs differ in exactly this way.[9] This can readily be done provided that the x-ray diffraction data from which the Patterson synthesis (2) has been made is on an absolute basis. Then, the total number of electrons within each peak is of the form z_1z_2 for the first member of each pair and $2z_1z_2$ for the second member of each pair, where z_1 and z_2 are the atomic numbers of atoms 1 and 2 in the crystal structure. For peaks in the symmetrical concentration loci of $R3$, $R\bar{3}$, $C3$ and $C\bar{3}$, this condition reduces to peaks of z^2 and $2z^2$, where z is the atomic number of an atom in the structure.

Thus, an incidental consequence of the conclusion that crystal structures are solvable from diffraction data is that all space groups can be distinguished from diffraction data except that one cannot distinguish between members of the enantiomorphous pairs. A rather similar but less general conclusion has been reached on other grounds. Wilson[15] has recently shown that the presence or absence of an inversion center (not detectable by observation of relative intensities, which results only in distinguishing the 11 centrosymmetrical crystal classes) can be discerned by a quantitative statistical analysis of all spectra produced by the crystal. Rogers[16] then pointed out that this additional feature could be used to modify the writer's diffraction symbols. When the possibility of distinguishing centrosymmetrical zones is taken into account, this permits distinguishing all but the 11 enantiomorphous pairs and the following four pairs of space groups:

$$\left\{ \begin{array}{l} I222 \\ I2_12_12_1 \end{array} \right. \qquad \left\{ \begin{array}{l} I23 \\ I2_13 \end{array} \right. \qquad \left\{ \begin{array}{l} C3lm \\ C3ml \end{array} \right. \qquad \left\{ \begin{array}{l} C\bar{3}lm \\ C3ml \end{array} \right.$$

Since these four pairs *can* be distinguished by the vector set method, the latter is more powerful. Of course, it has this greater power because the patterns themselves are examinable in the concentration loci, and because the loci are correlated with the lattice (which accounts for distinguishing the last two pairs above).

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[4] Laue, M., "Röntgenstrahlinterferenzen," *Physik. Z.*, **14**, 1075–1079 (1913).

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[6] Buerger, M. J., "The Application of Plane Groups to the Interpretation of Weissenberg Photographs," *Z. Krist.*, A91 (1935), especially pp. 287–288.

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[9] Buerger, M. J., reference 7, p. 2.

[10] Patterson, A. L., "A Fourier Series Method for the Determination of Interatomic Distances in Crystals," *Phys. Rev.*, **46**, 372–376 (1934).

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[13] It is possible to derive a vector set from any (2) (or to derive the fundamental set from (1)) by a pin-point-focussing process. To do this each F^2_{hkl} is divided by $f^2_{1,\ hkl}$ (or each F_{hkl} is divided by $f_{1,hkl}$), where $f_{1,\ hkl}$ is the scattering power of atom 1, selected to be pinpointed for spectrum hkl, at the temperature of the experimental determination of F^2_{hkl}.

[14] Pepinsky, R., "An Electronic Computor for X-ray Crystal Structure Analyses," *J. Applied Phys.*, **18**, 601–604 (1947).

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[16] Rogers, D., "Determination of Space Groups by Intensity Statistics, *Ibid.*, **2**, 342–343 (1949).

THE PHOTOGRAPHY OF ATOMS IN CRYSTALS

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Boersch[1] first called attention to the possibility of producing images of the atoms in crystals by using the diffraction from a grating corresponding to the reciprocal lattice. Shortly thereafter, Bragg[2] made a photograph by this method of the atoms in the crystal structure of the mineral diopside. Unfortunately, Bragg's "x-ray microscope" could only be applied to photographing that very limited class of crystal structures all of whose diffraction spectra had the same phase.

The writer immediately pointed out[3,4] that there exists a very large class of much-used two-dimensional Fourier summations in which the phases of all coefficients are identical, and that therefore these summations could be produced optically by diffraction from a weighted reciprocal lattice, the weighting being the Fourier coefficients. The new class of syntheses for which the optical method could be used includes the Patterson[5,6] synthesis, $P(xy)$, and the Harker[7] zero-level synthesis, $P(xy0)$. Furthermore, such syntheses were actually carried out by this optical method. More recently, the writer has also used the optical method for producing the real part of the Fourier transforms of molecules.

In one of these papers, the writer[4] also suggested that the method could be easily extended to the general Harker synthesis, $P(xyn)$, and could be extended to the photography of all crystal structures by the use of a simple device for controlling the phases of the diffraction spectra. This requires a collection of small pieces of mica cut from a single homogeneous mica cleavage. To use this method, a mica piece is placed in the path of the rays of any spectrum which requires a phase shift. To produce a particular phase shift of angle $r \cdot 2\pi$, it is only necessary to incline the mica piece so that the optical path through it is increased over that of the surrounding air by an amount $r\lambda$, where λ is the wave-length of the light used in the experiment.

The chief difficulty in practicing this method of phase control is to find mica which will furnish a cleavage flake of uniform thickness over a sufficiently large area so that the rather large number of pieces needed to control the phases of the many spectra (say, between 100 and 400) can be cut from it. Experimental work done in 1941 confirmed that this method of phase control was indeed feasible. Mica cleavages were tested for uniformity in thickness by examining them by reflection close to a broad source of monochromatic light. This has the effect of contouring the mica with interference lines which remain unbroken in regions where the thick-

ness does not vary. By this means of examination, areas of uniform thickness are easily mapped out on any mica cleavage. The inclination required to produce any required phase shift was found for any given mica sheet by setting up a two-hole diffraction "grating" with the piece of mica to be tested over one of them. Without the mica, the two holes produce a set of interference fringes. With the mica, the maxima are shifted in accordance with the phase shift produced by the mica. The drift in maxima can thus be correlated with the angle which the mica makes with the ray, and in this way a particular mica sheet of any thickness can be calibrated for phase-shift versus inclination.
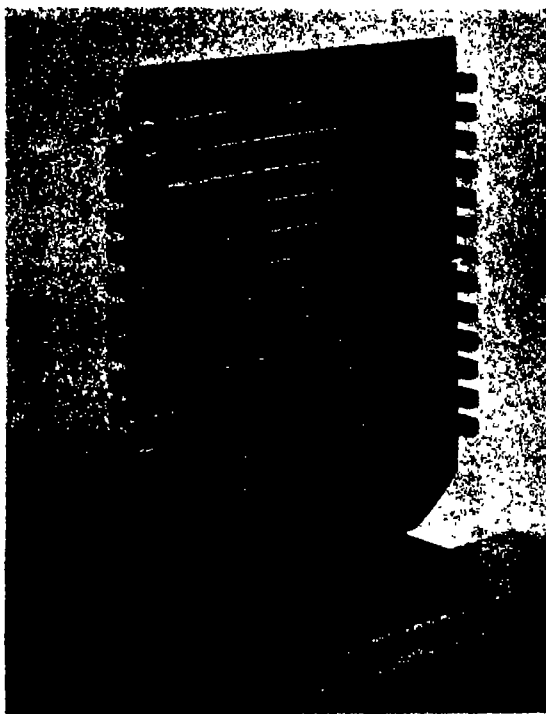
FIGURE 1

The war intervened in this experimentation, and its was discontinued until recently. In this new work, the writer was aided by a graduate student, Mr. Jay W. Lathrop, who selected and calibrated some large mica cleavages, and by Mr. John Solo, who executed the nice machining required for constructing the grating and the phase shifters.

The control of the phase of the diffraction was first applied to forming the image of the crystal structure of marcasite, FeS_2. The grating whose focused diffraction produced this image, and the method of controlling the

phases are indicated in figure 1.   The grating consists of holes drilled in a metal plate at points corresponding with the points of the reciprocal lattice of marcasite, and having translations a^* and b^*.   The amount of light passing through each hole of index hk is regulated by making the area of hole equal to $|F_{hk0}|$, i.e., to the absolute magnitude of the amplitude of the x-ray diffraction spectrum $hk0$ of marcasite.   The phase of the light emanating from each hole in the diffraction process is regulated, if necessary, by placing an inclined mica piece, held in a metal block at the correct inclination angle, in front of the hole.   Since marcasite is a centrosymmetrical crystal, the only phases which the spectra can have are 0 and π. The spectra having phase zero are not disturbed, but those having phase π
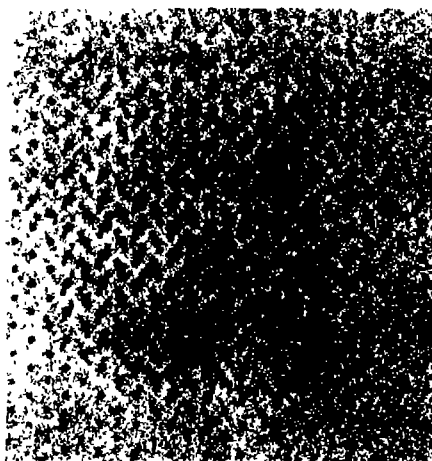
FIGURE 2	FIGURE 3
The pattern of atoms in the structure of marcasite, FeS$_2$, magnified 9 × 10^6 diameters.	The pattern of atoms in the structure of marcasite, FeS$_2$, magnified 2.1 × 10^7 diameters.

are properly phased by placing in front of each such hole a metal block. Thiry-two such blocks can be seen in figure 1.   On the sloping forward face of each block there is a mica piece so set that the slope provides an extra path difference of $(2n - 1)\dfrac{\lambda}{2}$ for the light.   This corresponds with shifting the phase of the spectrum by $(2n - 1)\pi$, which is equivalent to a phase shift of π.

The focused diffraction from this grating is shown in figures 2 and 3. Comparison of these photographs with the published diagrams of the crystal structure of marcasite[5] show that this is an accurate picture of the structure.   In fact, the blackening of figures 2 and 3 is a faithful repre-

sentation of the electron density in the structure. The patterns of these photographs are characterized by circular dark areas. The darkest of these are iron atoms with 26 electrons each, and the lighter circular areas are sulfur atoms with 16 electrons each. Careful study of the photograph shows a background of faint diffraction effects, caused by the fact that a limited number of spectra were used in forming the image. This corresponds with a limitation of aperture of the lens in ordinary microscopy. The magnification of figure 2 is about 9×10^6 diameters.

Another more complicated design of phase shifter has been prepared for crystal structures lacking a center of symmetry. In this more general design, the mica sheet is mounted on a rotating device so that the angle between the mica and the rays can be set at any value required to produce any phase shift for a particular spectrum.

The whole process of image formation by using diffraction from a weighted reciprocal lattice may be looked upon as essentially a two-stage,

$$M = \frac{T}{t} = \frac{D_2 \lambda_2}{D_1 \lambda_1}$$
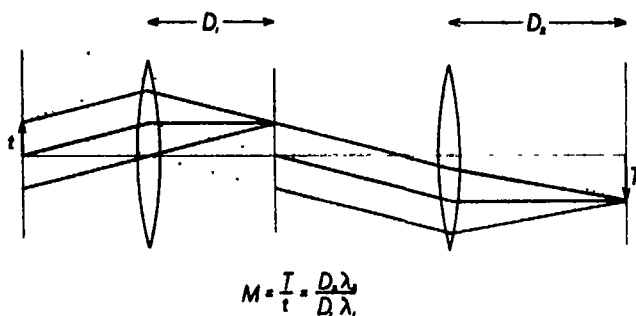
FIGURE 4

or two-wave-length, microscope. This is diagrammatically illustrated in figure 4. The diagram represents a simple optical system consisting of two lenses having focal lengths D_1 and D_2, respectively. The paths of rays ordinarily regarded as forming the image are indicated by dotted lines. But image formation can also be regarded as two diffractions in sequence. The focusing of these diffractions is illustrated by full lines. If the object is periodic (which simplifies the treatment by avoiding the explicit use of Fourier transforms) with period t, then the rays which are diffracted in the same direction by the object (i.e., rays which reach the lens as a parallel set) are focused at its right focus of the first lens to form a diffraction image. This is a collection of discrete spectra whose spacings are also periodic. This spacing is[4]

$$d^* = (D_1\lambda_1)\frac{1}{t}, \tag{1}$$

where D_1 and λ_1 are experimental constants. The reciprocity between d^* and t causes the discrete spectra in the diffraction image to be arranged on the points of the reciprocal lattice of the crystal which is used as an object.[4]

But the spectra of the diffraction image are also sources of rays. Those which reach the second lens as a parallel set are focused by it at its right focus. The first period of the final periodic image is formed by the first-order diffraction from the diffraction image. The length of this first period[4] in the final image is

$$T = (D_2\lambda_2)\frac{1}{d^*}. \tag{2}$$

Now, the magnification of the system is

$$M = \frac{\text{image size}}{\text{object size}} = \frac{T}{t}. \tag{3}$$

Substituting for t and T from (1) and (2), this magnification is

$$M = \frac{D_2\lambda_2}{D_1\lambda_1}. \tag{4}$$

In an ordinary optical system, the same light is used throughout. In this case (4) reduces to the familiar $M = D_2/D_1$, i.e., the image:object distance ratio. But if the wave-length is changed between diffraction stages, the further factor of λ_2/λ_1 enters into the magnification. Using some reasonable values (in centimeters) of (4), a magnification of

$$M = \frac{2 \times 10^2}{5} \times \frac{5 \times 10^{-5}}{7 \times 10^{-9}} = 3 \times 10^5 \text{ diameters} \tag{5}$$

can be achieved. In fact, these are the approximate actual values used for the instruments which produced the photographs of figures 2 and 3. This great magnification is not empty, since the resolving power depends on the wave-length of the first stage and the number of spectra collected in the first diffraction image.

While x-rays cannot be focused with lenses, the x-ray diffraction produced by a crystal can be arranged in the form of a reciprocal lattice by the precession camera,[5] just as if the diffraction image were actually produced by the first lens of figure 4. The constants in the denominators of (5) represent a 5-cm. crystal-to-film distance in the precession camera, and the wave-length of $\text{Mo}K\alpha$ x-radiation. The constants in the numerator of (5) are the focal length of the lens actually used in figure 4, and the wave-length of green light. This tremendous magnification is sufficient to enlarge the image of an atom so that it would be of the order of 0.03 mm. in diameter, which is big enough so that it can be seen conveniently by a

microscope.   Figures 2 and 3 were taken with a microscope of moderate magnification.

This description of microscopy can be epitomized by saying that the image is formed from the object by diffraction from the diffraction image of the object.   In other words, it is two-stage diffraction.   When it is caused to occur in two separate steps, with or without two different wavelengths, the phases of the diffraction spectra in the first diffraction image are lost.   It is this loss of phase which the phase shifters are required to supply.   If they are not supplied, a vector map of the object results instead of a simple image.   It is possible to decompose a vector map[10] into simple images, and when this is done, the phases required for the phase shifters may be learned.

This generalized theory of microscopy as two stages of diffraction is not new.   It was developed by the writer in 1939, in connection with the experimental development of the instrument, and was first presented at the January 10, 1941, meeting of the New York Academy of Sciences, again at the October, 1942, meeting of the Rochester Section of the American Optical Society, and on some other occasions.   What makes the matter of interest now is that it is a novel way of looking at the meaning of photographs of atoms in crystals, such as those shown in figures 2 and 3.

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STUDIES ON THE MECHANISM OF THE OXYGEN EFFECT ON THE RADIOSENSITIVITY OF TRADESCANTIA CHROMOSOMES*

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Previous experiments (Giles and Riley[1]) have demonstrated that the radiosensitivity of Tradescantia chromosomes, as measured by the occurrence of x-ray-induced aberrations in microspores, is markedly influenced by the amount of oxygen present. The frequencies of both interchanges and interstitial deletions observed four to five days following treatment are reduced if inflorescences are irradiated in gases such as nitrogen and helium and increased if exposures are made in pure oxygen instead of in air. On the basis of these experiments it was not possible to decide whether this effect of oxygen is exerted by way of the initial breakage mechanism, such that more breaks are produced by x-rays in the presence of oxygen, or whether the effect is on the recovery process, such that new reunions of broken ends are favored over restitutions. The present paper will discuss experiments performed to investigate certain aspects of this problem. Additional data will also be presented on the relation between aberration frequency and the percentage of oxygen present at the time of irradiation.

Experimental Methods.—Inflorescences of *Tradescantia paludosa* Anders. and Woodson, clone 5 (unless otherwise noted) of Sax were used. Aceto-carmine smear preparations of microspores at the first postmeiotic mitosis were made on the fourth and fifth days following irradiation and slides were scored for chromosomal aberrations—interchanges (dicentrics and centric rings) and interstitial deletions. In general, 50 or 100 cells from three to eight slides (each from a separate inflorescence) were scored and standard errors were calculated as previously. The same x-ray source was utilized—a Coolidge self-rectifying tube with tungsten target, operated at 250 kv and 15 ma. The inherent filtration was equivalent to 3 mm. of aluminum.

Irradiations were carried out as in earlier experiments in a lucite exposure chamber. However, in order to insure a more adequate control of the gas in the chamber and to facilitate a rapid removal or introduction of gas the experimental apparatus was redesigned. The new exposure chamber was placed directly in the x-ray machine and attached by pressure tubing and appropriate stopcock arrangements to a vacuum pump, a gas cylinder, and a mercury manometer. With this apparatus, all evacuations and introductions of gases could be performed directly with the inflorescences inside the exposure chamber. Evidence will be presented that the pre-exposure evacuations in a suction flask, as carried out in the earlier experiments, are unnecessary. Further, it is possible with this equipment to irradiate in a vacuum or under pressure and to introduce or remove gases during the period of irradiation. The presence of a manometer makes it possible to reproduce evacuation conditions and to detect possible unexpected changes of pressure in the system.

TABLE 1

COMPARATIVE EFFECTS OF VARIOUS PRETREATMENTS AND EXPOSURE-CHAMBER CONDITIONS ON THE FREQUENCY OF X-RAY INDUCED CHROMOSOMAL REARRANGEMENTS IN TRADESCANTIA MICROSPORES

400 r AT 50 r/MIN.

PRETREATMENT	EXPOSURE CONDITIONS	NO. CELLS	INTER-CHANGES	INTERCHANGES PER CELL	INTER-STITIAL DELETIONS	I. D. PER CELL
Buds preevacuated and helium admitted	Helium in chamber	226	55	0.24 ± 0.03	51	0.23 ± 0.03
Buds preevacuated and helium admitted	Air in chamber	400	280	0.72 ± 0.04	323	0.81 ± 0.05
None	Helium in chamber	300	73	0.24 ± 0.03	72	0.24 ± 0.03

Results and Discussion.—A preliminary series of experiments was performed to determine whether the preexposure evacuations in a suction flask, as carried out in earlier experiments, were necessary to remove air enclosed around the anthers by the sepals and petals of the buds. For these tests two sets of buds were evacuated five times in a flask and helium permitted to diffuse in before irradiation. One set was placed in the exposure chamber (the original chamber was used), which was then evacuated and helium admitted; the other set was placed in the exposure chamber in air. The third set of buds was not preevacuated, but placed directly in the exposure chamber, which was then evacuated and helium admitted. All three sets received 400 r at 50 r/min. (table 1). It is clear that the pretreatment has no effect on the frequency of aberrations, but rather that the gas in which the exposure is made is the important factor. Evidently evacuation in the exposure chamber is sufficient to effect gas exchange in the buds. On the basis of these results, preevacuation of buds with a water pump was discontinued. Further, with the new chamber

and a vacuum pump it was possible to evacuate directly to considerably lower pressures than in the earlier experiments.

In the original experiments comparative exposures were made at only three oxygen levels—in nitrogen or helium (no oxygen), in air (ca. 21% oxygen), and in pure oxygen. It seemed of considerable interest to obtain further data in order to determine the quantitative relation between aberration frequency and percentage of oxygen during irradiation. Consequently, two separate experiments were carried out (in one clone 5 was used, in the other, clone 3 of Sax) in which exposures to a single x-ray dose—400 r at 50 r/min.—were made with seven different percentages of oxygen in the lucite chamber. The oxygen percentages were as follows:

TABLE 2

EFFECT OF VARIOUS PERCENTAGES OF OXYGEN ON THE FREQUENCY OF CHROMOSOMAL ABERRATIONS IN TRADESCANTIA MICROSPORES

ALL X-RAY EXPOSURES OF 400 r AT 50 r/MIN.

OXYGEN PERCENTAGE	CLONE USED	NO. CELLS	NO. INTER- CHANGES	INTERCHANGES PER CELL	INTERSTITIAL DELETIONS	I. D. PER CELL
0	Clone 3	450	120	0.27 ± 0.02	104	0.23 ± 0.02
	Clone 5	400	113	0.28 ± 0.03	88	0.22 ± 0.02
2	Clone 3	284	73	0.26 ± 0.03	95	0.33 ± 0.03
	Clone 5	315	82	0.26 ± 0.03	77	0.24 ± 0.03
10	Clone 3	425	304	0.72 ± 0.04	305	0.72 ± 0.04
	Clone 5	200	152	0.76 ± 0.06	162	0.81 ± 0.06
21	Clone 5	350	322	0.92 ± 0.05	392	1.12 ± 0.06
(Air)	Clone 5	150	118	0.79 ± 0.07	116	0.77 ± 0.07
60	Clone 3	303	283	0.93 ± 0.06	364	1.20 ± 0.06
	Clone 5	200	181	0.91 ± 0.07	196	0.98 ± 0.07
100	Clone 3	250	249	1.00 ± 0.06	318	1.27 ± 0.07
	Clone 5	150	148	0.99 ± 0.08	159	1.06 ± 0.08
100 (at an absolute pressure of 1500 mm. Hg)	Clone 3	225	245	1.09 ± 0.07	279	1.24 ± 0.07

0% (irradiation in pure—99.8%—helium); 2% (+98% helium); 10% (+90% helium); 21% (air); 60% (+40% helium); 99.5% (pure oxygen from a commercial cylinder); and pure oxygen at an absolute pressure of 1500 mm. of mercury (approximately 760 mm. above normal atmospheric pressure at Oak Ridge). Before each exposure, inflorescences were placed in the chamber, which was then evacuated to approximately 1 to 2 mm. of mercury, and the particular gas or gas mixture admitted. This procedure was repeated five times. Following irradiation, the inflorescences remained in the chamber for approximately ten minutes in the same gas and were then removed to air. The results of these experiments are presented in table 2 and figure 1. There is good agreement between the two

experiments except for the two points in air, where, for some unexplained reason, the values obtained for clone 3 are considerably higher than expected. The data indicate that there is a very rapid rise in aberration frequency between 2 and 20% oxygen, after which a gradual increase apparently occurs. The significance of the fact that essentially the same aberration frequencies were obtained in exposures made in pure (99.8%) helium and 2% oxygen (+98% helium) is not yet clear. This may mean that not all of the dissolved air is removed from the tissues by the evacuation procedure. It is also desirable to check further the accuracy of the reported percentage of oxygen in the gas mixture used (obtained from a
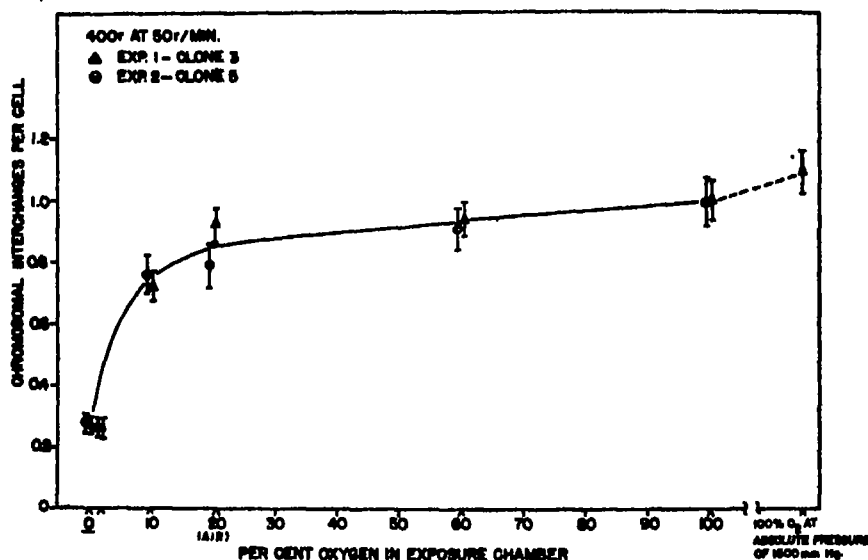
FIGURE 1

Relation between percentage of oxygen in exposure chamber and frequency of chromosomal interchanges per cell in Tradescantia microspores. All exposure to one x-ray dosage—400 r at 50 r/min. Two separate experiments, one with clone 3 and one with clone 5, as indicated.

commercial source). The general problem of the effect of oxygen during irradiation at low oxygen tensions is being investigated further.

The major problem requiring further investigation was concerned with the mechanism of the oxygen effect in increasing aberration frequencies— whether this effect resulted from a higher initial production of chromosome breaks, or from a relative increase in new reunions as opposed to restitutions of broken ends during the recovery process. It is clear from the earlier studies of Sax,[2] Marinelli, Nebel, Giles and Charles,[3] and Lea and Catche-

side,[4] that in Tradescantia there is an appreciable time interval between the production of a break and its disappearance, either by restitution or new reunion (interchange). The average time of restitution for the majority of the breaks has been estimated by Lea[5] to be about four minutes. Thus it would appear to be experimentally feasible to determine whether the oxygen effect is on initial breakage or on recovery if these two processes can be made to take place under different conditions with respect to the presence or absence of oxygen. The following series of comparative exposures (all at a single constant dosage of 300 r at 300 r/min.) was

TABLE 3

EXPERIMENTS ON THE MECHANISM OF THE EFFECT OF OXYGEN IN INCREASING THE RADIOSENSITIVITY OF TRADESCANTIA CHROMOSOMES. FOR FURTHER DISCUSSION, SEE TEXT.

ALL EXPOSURES, 300 r AT 300 r/MIN.

SERIES NO.	PRE-TREATMENT CONDITIONS	EXPOSURE CONDITIONS	POSTTREATMENT CONDITIONS	NO. CELLS	INTERCHANGES PER CELL	INTERSTITIAL DELETIONS PER CELL
1	Buds in vacuum	Vacuum	Vacuum—10 min.	880	0.12 ± 0.01	0.11 ± 0.01
2	Buds in vacuum	Vacuum	Oxygen introduced (within 3 secs.) to 1500 mm. Hg—10 min.	700	0.09 ± 0.01	0.10 ± 0.01
3	Buds in oxygen	Oxygen at 1500 mm. Hg	Oxygen at 1500 mm. Hg —10 min.	150	0.70 ± 0.07	0.83 ± 0.07
4	Buds in oxygen	Oxygen at 1500 mm. Hg	Evacuation (within 25 sec.); vacuum 10 min.	200	0.72 ± 0.06	0.85 ± 0.07
5	Buds in vacuum	1st 30 sec., vacuum. 2nd 30 sec., oxygen introduced (within 3 sec.) to 1500 mm. Hg	Evacuation (within 25 sec.), vacuum —10 min.	350	0 39 ± 0.03	0.50 ± 0.04
6	Buds in oxygen	1st 30 sec., oxygen at 1500 mm. Hg. 2nd 30 sec., evacuated (within 25 sec.) to 1 to 2 mm. Hg	Oxygen introduced (within 3 sec.) to 1500 mm. Hg— 10 min.	518	0.61 ± 0.03	0.59 ± 0.03

accordingly carried out. The exposure conditions about to be described are summarized in table 3. In series 1 and 2 buds were evacuated for five minutes at 1 to 2 mm. of mercury and irradiated in vacuum. Series 1 was maintained in vacuum in the exposure chamber for ten minutes following irradiation. In series 2 pure oxygen was introduced into the exposure chamber to an absolute pressure of 1500 mm. of mercury immediately following irradiation. The introduction of oxygen to this pressure was effected within three seconds following cessation of the irradiation and the buds were maintained in oxygen for ten minutes. In series 3 and 4

buds were placed in oxygen (by the usual procedure for evacuation and introduction of gas) at an absolute pressure of 1500 mm. of mercury and irradiated in oxygen. Series 3 was maintained in oxygen for ten minutes after irradiation. In series 4 the chamber was evacuated immediately following irradiation. This evacuation to 1 to 2 mm. of mercury was accomplished within 25 seconds following the cessation of the irradiation and the vacuum was maintained for ten minutes. In series 5 buds were evacuated as in series 1 and 2 and irradiation was commenced with the buds in a vacuum. At the end of 30 seconds of exposure, oxygen was introduced into the chamber, without interrupting the irradiation, to an absolute pressure of 1500 mm. of mercury (within three seconds) and after the cessation of the total irradiation time of 60 seconds, the chamber was immediately evacuated to 1 to 2 mm. of mercury (within 25 seconds) and the buds kept in vacuum for ten minutes. In series 6 buds were placed in oxygen as in series 3 and 4 and irradiation was commenced with the buds in oxygen. At the end of 30 seconds of exposure the chamber was evacuated, without interrupting the irradiation, to 1 to 2 mm. of mercury (within 25 seconds) and after the cessation of the total irradiation time of 60 seconds oxygen was reintroduced to an absolute pressure of 1500 mm. of mercury (within three seconds). The exposure of 300 r/min. for one minute was selected to make the total time of exposure as short as feasible compared to the average time for restitution, since restitution takes place during the period of irradiation also. The data obtained from these exposures are presented in table 3.

It is clear from the first comparison (series 1 and 2) that the addition of oxygen immediately after irradiation does not increase the frequency of aberrations. Such an increase would be expected if there were an effect of oxygen on the reunion process. Nor does the removal of oxygen (series 3 and 4) after irradiation result in a lower aberration frequency. In both comparisons the observed aberration frequency apparently depends on the presence or absence of oxygen at the time of irradiation. The additional experiments were included to test this point further and also to exclude the possibility that the addition or removal of oxygen (to or from the cells themselves) after irradiation was not accomplished rapidly enough to detect an effect on the reunion process if such an effect existed. In series 5, it is evident that the addition of oxygen during irradiation results in a marked increase in aberration frequency. There is apparently an almost immediate entrance of a considerable amount of oxygen into the cells of the anthers at the beginning of the final 30 seconds of irradiation (the period of three seconds indicated is the time required to introduce oxygen to a pressure of 1500 mm. of mercury; considerably less time is probably required for the introduction of sufficient oxygen to produce an almost maximal increase in aberration frequency). In series 6 there is an appreciable decrease in aberration frequency accompanying the removal of

oxygen during the last 30 seconds of radiation.   The fact that this decrease is not as marked as the increase noted on the addition of oxygen in series 5 appears to be quite reasonable, since a considerably longer period is required to evacuate the chamber than to introduce oxygen.

On the basis of the data which have been presented it is clear that the postirradiation presence or absence of oxygen has no effect on the recovery process.   It seems reasonable to conclude also that there is no effect of oxygen on the recovery process occurring during irradiation, which in these experiments occupies a relatively small fraction of the total recovery period. Pretreatment of buds in the presence or absence of oxygen has no effect; oxygen must be present during the actual X-ray exposure to produce an increase in aberration frequency.   Furthermore, the experiments in which oxygen is introduced during irradiation indicate that its effect is immediate. It thus appears that the effect of oxygen must be exerted on the breakage mechanism.   However, it seems probable that this effect is actually an indirect one.   The most likely hypothesis seems to be that when dissolved oxygen is present during irradiation in the largely aqueous medium in cells, some substance resulting from the radiodecomposition of water containing oxygen is formed which causes an increased aberration frequency.   On the basis of their experiments with *Vicia faba*, Thoday and Reed[6] suggest that this substance may be hydrogen peroxide.   This possibility is supported by the results obtained in the present experiments relating aberration frequency to percentage of oxygen at the time of irradiation.   This relation (fig. 1) is generally similar to that for the yield of hydrogen peroxide when water containing increasing concentrations of oxygen is subjected to X rays (Bonet-Maury and Lefort[7]).   If it is assumed that hydrogen peroxide is in fact the substance indirectly responsible for the increased aberration frequency obtained in oxygen, it appears likely that such an increase would result from a higher frequency of initial breakage, rather than from an effect on the recovery process.   This conclusion is supported by the observations of Baker and Sgourakis[8] that oxygen increases the yield of X-ray induced sex-linked lethal mutations in *Drosophila*, where there is no evidence that a recovery process is involved.   However, the possibility cannot yet be excluded that in Tradescantia an intermediate radiation product such as hydrogen peroxide might produce an effect by modifying the behavior of broken ends, themselves produced by direct radiation action, and thus influence the restitution process.

Summary.—Further experiments have been performed on the effect of oxygen in increasing the radiosensitivity of Tradescantia microspore chromosomes.   Exposures of inflorescences to a single constant x-ray dose, but in atmospheres containing seven different percentages of oxygen indicate that there is a rapid rise in aberration frequency between 2 and 21% oxygen, with a gradual increase thereafter.   Further studies are being made to clarify the effect of oxygen at levels between zero and two

per cent.   Other experiments have been performed to determine whether the oxygen effect is exerted by way of the initial breakage mechanism or on the reunion process.   These consisted of comparative exposures to a single dose of 300 r in one minute of inflorescences in a vacuum or in oxygen with the addition or removal of oxygen either immediately after or during part of the irradiation period.   In addition, buds were pretreated in the presence and absence of oxygen before exposure to X rays.   These experiments show that the presence of oxygen during the actual exposure to X rays rather than during the pre- or postirradiation period is the important factor, thus indicating that oxygen alone does not influence the recovery process.   It seems likely that the oxygen effect is an indirect one, resulting from the production during irradiation in oxygen of some substance such as hydrogen peroxide.   Although it appears probable that the effect of such a substance on aberration frequency would result from an increased production of chromosome breaks, the alternative possibility, that such a substance might modify the restitution process, cannot yet be excluded.

Acknowledgment.—The authors wish to acknowledge the assistance of Lucille McMichael Fairchild in certain of these experiments.

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THE ORIGIN AND BEHAVIOR OF MUTABLE LOCI IN MAIZE

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In the course of an experiment designed to reveal the genic composition of the short arm of chromosome 9, a phenomenon of rare occurrence (or recognition) in maize began to appear with remarkably high frequencies in the cultures.   The terms mutable genes, unstable genes, variegation,

mosaicism, mutable loci or "position-effect" have been applied to this phenomenon. Its occurrence in a wide variety of organisms has been recognized. The most extensive investigations of this phenomenon have been undertaken in *Drosophila melanogaster*.[1] In this organism, the conditions associated with the origin of genic instability have been well defined. The part played by the heterochromatic materials of the chromosomes, in inducing and controlling the type of variegation and its time and frequency of occurrence, has been established. It has not been generally recognized that the instability of genic expression in other organisms may be essentially the same as that occurring in Drosophila.

As stated above, a large number of mutable loci have recently arisen in the maize cultures and are continuing to arise anew. The loci affect variegation for many different kinds of plant characters, each locus being concerned with a particular character or occasionally several characters. Some of these loci are *c, yg₂, wx, a₂, y, pyd,* which are well-investigated units in maize.[2] Others involve previously unknown genetic units. The same types of genic instability appearing in the maize cultures have been described in many other organisms. The behavior of these new mutable loci in maize cannot be considered peculiar to this organism. The author believes that the mechanism underlying the phenomenon of variegation is basically the same in all organisms. The reasons for this conclusion will be made apparent in the discussion.

The initial appearance of the burst of newly arising mutable loci occurred in the progeny coming from the self-pollination of about 450 plants which had each undergone a series of events in their early development where the short arm of chromosome 9 was subjected to drastic structural modifications. These events took place during the "chromosome type" of breakage-fusion-bridge cycle.[3] The modifications that this mechanism produces are: one or more duplications of segments of the short arm, deficiencies of one or more segments of various lengths, structural modifications of the heterochromatic knob substance, duplications of the knobs with or without structural modifications, and various combinations of these several types of modifications. The chromosome complement of over 150 of these plants were examined at pachytene to determine the nature of the structural modifications that had occurred. In addition to the modifications of the short arm of chromosome 9 listed above, some of the plants had other modifications, many of which are particularly significant because they involve the substances in the chromosome that are believed to be responsible for the origin and behavior of mutable loci—the heterochromatic knobs and centromeres. Altogether, 48 such structural modifications have been analyzed, most particularly in the above-mentioned plants but also in some other plants that had received a chromosome 9 with a newly broken end. Fourteen involved modifications of chromo-

some 9 other than those listed above (telocentric chromosomes, isochromosomes, extra chromosomes 9 with particular modifications, etc.). Four arose from fusion of the centromere of chromosome 9 with the centromere of another chromosome. Four resulted from fusion of the knob substance of the short arm of chromosome 9 with the centromere of chromosome 9. Twenty-four resulted from fusions of the knob substance of the short arm of chromosome 9 with other regions in the chromosome complement: eighteen were with other knobs or with regions very close to these knobs, four were insufficiently analyzed as to the positions of the fusion, and two did not involve a known knob region. In two cases, inversions were present in other chromosomes. The regions involved were the knob and centromere in one of these chromosomes and the nucleolus organizer and the centromere in the other chromosome. There can be no question that these "spontaneous translocations" are nonrandom with respect to the location of the breaks and fusions. The heterochromatic knob and centromere regions are mainly involved.

In the cultures arising from self-pollination of the plants that had undergone the chromosome type of breakage-fusion-bridge cycle in their early development, about 40 different mutable loci were recognized. The majority of such mutable loci could not have been present in the parents of these plants, for the stocks from which they arose had been under investigation for some years without showing evidence of the presence of such a large number of unstable loci. It was concluded, therefore, that either some part of the mechanism concerned with the breakage-fusion-bridge cycle or some of the structural modifications resulting from it were responsible for conditions that produced this burst. That some of the mutable loci were located in or associated with chromosome 9 was realized in the first tests. Other mutable loci, on the other hand, did not show any obvious association with chromosome 9.

The mutable loci fall into two major classes: (1) those that require a separate activator factor for instability to be expressed, and (2) those that are autonomous with respect to the factor that controls the onset of mutability. They also may be subdivided on a quite different basis. This is related to the types of expression of the mutations that occur. The following types are present: (a) Changes from the mutant to, or close to, the wild-type expression. After such a mutation, the locus may be permanently stabilized. It may no longer show evidence of the instability phenomenon. (b) A second group, similar to (a) except that the mutation to wild-type does not produce stability of the locus. The wild-type-producing locus, in turn, may mutate to give the recessive expression. (c) A third type where the mutations give rise to a series of alleles of the affected loci. These alleles are distinguished by different degrees of quantitative expression of the normal phenotype. Most of these are

relatively stable; only rarely does instability again appear. (d) A fourth type, similar to (c). Most of the alleles, however, are not stable for they, in turn, can mutate in the direction of a higher or lower grade of quantitative expression of the phenotype. Mutable loci showing these different types of expression of mutation are found in both the major classes, that is, in the activator-requiring class and in the autonomous class.

The accumulated observations and data from a study of a number of these mutable loci are so extensive that no short account would give sufficient information to prepare the reader for an independent judgment of the nature of the phenomenon. It is realized that this is unfortunate. Manuscripts giving full accounts of some of this phenomenon are in preparation. Since this task will require much time to fulfill, the author has decided to present this short account of the general nature of the study, and the conclusions and interpretations that have been drawn. In this account only short summaries will be given of some of the pertinent information that has led to the conclusions to be presented. These conclusions are concerned with the origin of mutable loci, the events occurring at these loci that result in a change in phenotypic expression, the reasons for changes in the frequency of visible mutations at these loci, the factors controlling the time when mutations will occur, the production of mutations at the a_1 locus in maize without Dt being present, and heterochromatin as the probable controlling factor.

A fortunate discovery was made early in the study of the mutable loci which proved to be of singular importance in showing the kinds of events that are associated with their origin and behavior. A locus was found in the short arm of chromosome 9 at which breaks were occurring in somatic cells. The time and frequency of the breakage events occurring at this Ds (Dissociation) locus appeared to be the same as the time and frequency of the mutation-producing events occurring at some of the mutable loci.[4] An extensive study of the Ds locus has indicated the reason for this relationship and has produced the information required to interpret the events occurring at mutable loci. It has been concluded that the changed phenotypic expressions of such loci are related to changes in a chromatin element other than that composing the genes themselves, and that mutable loci arise when such chromatin is inserted adjacent to the genes that are showing the variegated expression. The events occurring to this inserted chromatin are reflected in a changed expression of the neighboring genes, or sometimes in a loss of these genes. It is the inserted material that is undergoing the "mutational" events. The Ds locus is composed of this kind of material.

Various types of alterations are observed as the consequence of events occurring at the Ds locus. Some of these alterations resemble the effects produced by x-rays, ultra-violet light, chemicals, etc. They involve

chromosome breakage and fusion. The breaks are related, however, to events occurring at this one specific locus in the chromosome—the *Ds* locus. The *Ds* designation was given to this locus because the dissociation, now known to be related to dicentric and associated acentric chromatid formation, was recognized before the other events occurring at *Ds* had been disclosed. Some of the events occurring at *Ds*, when considered without reference to all the known events, would not by themselves suggest that changed conditions at this locus are associated with a breakage-inducing phenomenon. All of them can be explained, however, by the assumption that one kind of alteration of the inserted chromatin (the chromatin of the *Ds* locus) takes place, and that the various kinds of changes observed represent consequences of this one altered condition. This condition is assumed to be a stickiness of the materials composing the *Ds* locus, which arises only at precise times in the development of a tissue. The control of the timing of this changed condition will be considered shortly. The reasons for assuming the change to be a stickiness will be obvious from the following list of known events that involve the *Ds* locus. These are: (1) Dicentric chromatid formation with fusion of sister chromatids at the location of *Ds*. This is accompanied by formation of an acentric fragment composed of the two sister segments of this arm, from *Ds* to the end of the arm. (2) Loss of detectable *Ds* activity without visible alteration of the chromosome. In some cases, the loss of *Ds* activity is presumably due to loss of the locus itself. (3) Deletions of chromatin segments of various lengths adjacent to *Ds*, usually with concomitant loss of *Ds* activity but occasionally without loss of this activity. (4) Reciprocal translocation involving chromosome 9 in which one breakage point is at *Ds*. (5) Duplications of segments of chromosome 9, inversion or ring chromosome formations involving chromosome 9 with one break at the *Ds* locus. (6) Transposition of *Ds* activity from one position to another in the chromosomal complement with or without an associated gross chromosomal rearrangement. (7) Changes at the *Ds* locus itself which result in precise changes in the relative frequency of occurrence of the above types of events in future cell and plant generations. This last event, which is of considerable importance, has been termed "change in state" of the *Ds* locus. From a study of the progression of changes in state of *Ds* through cell and plant generations, it appears that the various states may reflect the quantity of the inserted chromatin, the *Ds* loci with larger quantities of this material showing a high frequency of consequences (1), (3), (4), (5), and (6) above, and those with less of this material showing high frequencies of consequence (2) above.

It is from the transpositions of *Ds* that some of the new mutable loci may arise. The mechanism of transposition has received considerable study. Some cases of transposition of *Ds* are associated with a gross

chromosomal rearrangement. In these cases, two chromosome breaks occur to give rise to the rearrangement; one break marks the known position of *Ds* in the chromosome, before the rearrangement occurred, and the second break marks the new position of *Ds* activity. Sister chromatids are affected at each of these two positions of breakage. It has been determined for several of these cases that the appearance of *Ds* activity at the new position most probably arose at the time of origin of the gross chromosomal rearrangement. One case of transposition of *Ds* has been of particular importance because it illustrates how new mutable loci, associated with changes in genic expression, can arise. This transposed *Ds* locus appeared in a single gamete of a plant carrying chromosomes 9 with the dominant *C* allele. This gamete carried a *Ds* locus that had been transposed from a known position in the chromosome 9 to a new position in the same chromosome. The chromosome having *Ds* at this new position was morphologically normal in appearance. This new position of *Ds* corresponded to the known location of *C* (*C*, colored aleurone, dominant to *c*, colorless aleurone). All of the above-enumerated events were now occurring at this new position. Significantly, the appearance of *Ds* activity at this new location was correlated with the disappearance of the normal action of the *C* locus. The resulting phenotype was the same as that produced by the known recessive, *c*. It has been determined from previous studies that a deficiency of the *C* locus will give rise to a *c* phenotype. That the *c* phenotype in this case was associated with the appearance of *Ds* at the *C* locus, and was not due to a deficiency, was made evident because mutations at this locus from a *c* to a full *C* phenotypic expression occurred. It could be shown that when *C* action reappeared, the *Ds* action concomitantly disappeared from this locus. The restored action of *C* was permanent; no further *Ds*-type events occurred at this *C* locus. In most cases, the event giving a restored *C* action did not result in an altered morphology of chromosome 9. Loss of *Ds* activity without concomitant structural alterations of the chromosome result from event (2) above.

The other enumerated events associated with *Ds* activity were also occurring at this mutable *c* locus. The dicentric chromatid formations were not associated with the appearance of a *C* phenotype, suggesting that the inserted inhibiting material composing *Ds* may be situated proximal to the *C* locus. Several cases of transposition of *Ds* from this location to still another location in the short arm of chromosome 9 were recognized. In each case, a restored *C* action was associated with a disappearance of *Ds* activity at the *C* locus and the appearance of *Ds* activity at the new position. The changes in state of *Ds* at this mutable *c* locus (event (7) above) are particularly significant since it has been determined that a specific change in state of *Ds* is often accompanied by a specific change in the frequency of *c* to *C* mutations.

The origin and behavior of this mutable *c* locus has been interpreted as follows: Insertion of the chromatin composing *Ds* adjacent to the *C* locus is responsible for complete inhibition of the action of *C*. Removal of this foreign chromatin can occur. In many cases, the mechanism associated with this removal results in restoration of the former genic organization and action. The *Ds* material and its behavior are responsible for the origin and the expression of instability of the mutable *c* locus. The mutation-producing mechanisms involve only *Ds*. No gene mutations occur at the *C* locus; the restoration of its action is due to the removal of the inhibiting *Ds* chromatin. The possible nature of the inserted material will be considered later.

In the cultures having *Ds*, other mutable loci continue to arise. They show types of behavior similar to that described for the mutable *c* locus. This mutable *c* locus (called *c-m1* because it was the first of the mutable *c* loci isolated in these cultures) belongs to the (*a*) group of mutable loci. In some of the progeny of the original self-pollinated cultures, other mutable *c* loci have arisen from previously normal *C* loci. One of these, *c-m2*, shows the type (*c*) expression of variegation, which differs markedly from that shown by *c-m1*. A wide range of quantitative expression, for at least two different reactions associated with aleurone pigment formation, appears as the consequence of various mutations at this locus. The intermediate alleles, full wild-type alleles and some alleles showing even stronger phenotypic expressions than the wild-type from which it arose, are produced by mutations at *c-m2*. The mutations are often expressed as twin sectors, the depth of color in one sector being greater than that in the sister sector. These twin sectors may reflect a single mutation-producing event at the *c-m2* locus that involved both sister chromatids. It has also been determined that chromosome breakage may occur at this locus.

The phenotypic expressions resulting from mutations of *c-m2* and *c-m1* are clearly quite different. That this difference may be related to differences in the inserted chromatin is suggested by the appearance of a mutable *wx* locus arising from a *Wx* locus in a gamete of a plant carrying *c-m2* (*Wx*, starch of endosperm stains blue with iodine; *wx*, recessive allele, starch stains red with iodine; located in short arm of chromosome 9, proximal to *C*). The type of variegation expressed by this mutable *wx* locus (*wx-m1*) is strikingly similar in all respects to that occurring at *c-m2*. It could not be determined in this case that transposition to the *Wx* locus of the same inhibiting substance that induced *c-m2* had occurred. Such an event is suspected from the known transposition capacities of this material.

In this report, *Ds*, *c-m1*, *c-m2* and *wx-m1* have been used as illustrations of newly arising mutable loci because all of them require an activator and

all respond to the same activator. This activator has been designated
Ac. Extensive studies of *Ac* have shown that it is inherited as a single
unit. It shows, however, a very important characteristic not exhibited
in studies of the inheritance of the usual genetic factors. This charac-
teristic is the same as that shown by *Ds*. Transposition of *Ac* takes place
from one position in the chromosomal complement to another—very often
from one chromosome to another. Again, as in *Ds*, changes in state may
occur at the *Ac* locus   These changes in state are of two main types:
either changes that resemble the known effects produced by different
doses of the *Ac*-locus from which it was derived, or changes that result in
a decidedly altered time of action and dosage response of *Ac*. *Ac* may be
detected and its action studied by observing the mutations occurring at
the mutable loci requiring its presence for mutability to be expressed.
It should be emphasized that when no *Ac* is present in a nucleus, no muta-
tion-producing events occur at *c-m1*, *c-m2* or *wx-m1;* nor are any chromo-
some breakage events detected at *Ds*, for no such events occur. As an
example of this interaction it may be stated that *c-m1* has been maintained
in cultures having no *Ac* locus for several generations, and has given com-
pletely colorless aleurone with no evidence of *c* to *C* mutations. Similarly,
the various quantitative alleles arising from mutations of *c-m2* or *wx-m1*
may be maintained without giving mutations, if *Ac* is removed from the
nucleus by appropriate crosses. Thus a series of stable recessive mutations
or stable alleles of a mutable locus may be isolated and maintained (if the
chromosome complement is normal, see below). When *Ac* is returned to
the nucleus, however, instability may again appear.

   The dosage action of *Ac* may be studied in the diploid plant or in the
triploid endosperm tissue of the kernels. When marked dosage effects
are produced by a particular state of *Ac*, they are registered alike in both
the plant and the endosperm tissues; the higher the dose of *Ac*, the more
delayed is the time of occurrence of mutations at the *Ac*-controlled mutable
loci. *Ac* determines, therefore, not only the mutation process at these
mutable loci but also the time at which the mutations occur, the different
states of *Ac* giving different times of occurrence in 1, 2 or 3 doses. The
action of *Ac* on the mutable loci it controls has been described. It is
believed that this action produces a stickiness of the inhibiting materials
adjacent to the affected loci. With reference to *Ds*, the observed conse-
quences of this stickiness have been enumerated. This physical change
probably takes place in the inserted inhibiting materials at all the *Ac*-
controlled mutable loci at the same time in the same cell. This latter
conclusion rests on the observation that mutations occur concomitantly
at two or more *Ac*-controlled mutable loci when these are present in the
same nucleus. The similarity in the type of inheritance and the behavior
of *Ds* and *Ac* has been indicated above. Another similarity is that changes

in state, loss or transposition of *Ac* occur at the same time that changes take place at the *Ac*-controlled mutable loci. It would appear that the changes in the physical properties of the specific inhibiting chromatin at the mutable loci and at *Ac* itself are of the same nature, and that all are expressions of the primary genetic action of the material composing *Ac*. It is suspected that *Ds* and *Ac* are composed of the same or similar types of material. The possible composition of this material will be considered · shortly.

The study of *Ac* and the *Ac*-controlled mutable loci has made it possible to interpret the many patterns of variegation exhibited by mutable loci. The variegated pattern is an expression of the time and frequency of occurrence of visible changes in the phenotype. The frequency of appearance of a visible mutation need not reflect the frequency of the events that occur at a mutable locus, as the study of *c-m1* has clearly revealed. The visible mutations reflect only the frequency of one or several particular consequences of one primary type of event occurring to the inhibiting material adjacent to the affected gene. The changes in state of this inhibiting material that arise as one of the consequences of the primary event, lead to changes in the relative frequency of the consequences of this event when it again occurs in future cell and plant generations. Such changes in state are reflected either in increases or decreases in the relative frequency of appearance of visible mutations. The study of *Ac* has indicated the nature of the control of the time when the mutations will occur at these mutable loci. The different doses of *Ac* together with the changed states of *Ac* control the time of occurrence of these mutations. The changes in time of occurrence of visible mutations are thus reflections of changes in dosage or changes in state of *Ac*.

The mutable loci that require no activator show the same kinds of expression of variegation as do the activator-requiring mutable loci. It has been shown that the changes occurring at *Ac* are much the same as those occurring at *Ds*. Thus, *Ac* or *Ac*-like loci, could be responsible for the origin of new mutable loci when transposed to a position adjacent to a gene whose inhibited action could be detected by a visible change in phenotype. Dosage action could be exhibited by such autonomous mutable loci, as well as various "changes in state," reflected by changes in the phenotypic expression and the time and frequency of occurrence of visible mutations of the affected genes. The study of the behavior of *Ds* in its several states makes it possible to reinterpret the variegation patterns in Drosophila, which in some cases appear to be associated with loss of segments of chromosomes and in other cases appear to be associated with changes in the degree of action of the genes involved. It also makes it possible to interpret the reported "position-effect" in Oenothera, because the events responsible for the changes in phenotype and the appearance of

duplications and deficiencies in this organism appear to be the same or similar to those described for Ds in maize.[5]

The possible composition of Ac may now be considered. Until recently, the investigation was not focused on this problem. It is believed, however, that this material is probably heterochromatin. This statement is based, in part, on the evident homologies in the expression of variegation in maize and Drosophila, but is more convincingly suggested by the results of a preliminary experiment focused on the induction of mutations at the a_1 locus in maize when the known Dt (Dotted) locus is absent. The action of Dt in chromosome 9 on the a_1 locus in chromosome 3 is very much the same as the action of Ac on the mutable loci it controls.[6] The similarities are too great to be dismissed as being due to causally unrelated phenomena. The Dt locus activates the a_1 locus; mutations to higher A_1 alleles occur (A_1, colored aleurone; a_1, colorless aleurone, recessive to A_1). Without Dt in the nucleus, a_1 has been shown to be completely stable. Dt is located in the *heterochromatic* knob terminating the short arm of chromosome 9. The suspicion is immediately aroused: Is Dt action caused by some modification of the heterochromatic knob in chromosome 9? If so, could this modification be produced anew by subjecting a chromosome 9 to the breakage-fusion-bridge cycle? Would the effective alterations of the knob arise directly because of the induced changes, or would they be produced secondarily by some other induced structural alteration, either within the short arm of chromosome 9 or elsewhere, that would upset, in some way, the normal functioning of the knob substance and thus bring about an alteration in its action? This last question is pertinent because some of the structural alterations in Drosophila appear to affect the functioning of the centrically placed heterochromatin. For example, some of the Minutes bring about chromosome elimination and "somatic-crossingover," both of which may well be related to adhesions of specific heterochromatin that occur at certain times in development.[7] To answer the above questions, plants homozygous for a_1 and having no Dt locus (designated dt by Rhoades) were crossed by plants similarly constituted with reference to a_1 and dt but carrying a rearrangement of the short arm of chromosome 9 that would introduce a chromosome 9 with a newly broken end into many of the primary endosperm nuclei in the given cross.[8] Breakage-fusion-bridge cycles involving such a chromosome 9 with a newly broken end would occur during the development of the kernels. Some of these broken chromosomes 9 would carry a knob, and this knob could then be subjected to modifications as a consequence of the breakage events. If some of these modifications gave rise to the same conditions that were present at Dt, mutations from a_1 to A_1 could appear in some of the kernels resulting from the cross. A large number of crosses of this type were made. The results were positive with respect to inducing mutations of a_1 to A_1. A small

number of the kernels resulting from these crosses showed mutations of a_1 to A_1. Often, only a single small A_1 spot was present on the kernel. Several of the kernels, however, had a pattern of mutations of a_1 to A_1 that was indistinguishable from that produced by Dt. These kernels could not have arisen by contamination, for stocks with the known Dt locus had never been obtained and thus no plants with this locus could have been present in the field. Furthermore, the stock having a_1 and dt, originally obtained from Rhoades, had been grown for several years. A number of sib crosses were made each year and no mutations of a_1 to A_1 were observed in the kernels on these ears.

The facts (1) that Dt is located in the heterochromatic knob of chromosome 9, (2) that the effect it produces can be recreated by subjecting chromosome 9 to the breakage-fusion-bridge cycle, (3) that Ac appeared in stocks that had undergone this cycle, and (4) that Ac and Dt are alike in their respective actions, all point to heterochromatin as the material composing Ac. The burst of new mutable loci which appeared in the self-pollinated progeny of plants that had been subjected to the chromosome type of breakage-fusion-bridge cycle becomes comprehensible if it is considered that the alterations in the quantity or structure of heterochromatic elements during this cycle were primarily responsible for the initial appearance of these mutable loci. This report has shown that, once such loci arise, other mutable loci arise through transposition of the inhibiting chromatin substances to other loci which in turn become mutable.

Why should altered heterochromatin be responsible for initiating such a chain of events? To answer this question, attention must be centered on the action of heterochromatin in the normal nucleus. That it is associated with the exchange of materials between nucleus and cytoplasm has been indicated.[9] Changes in quantity, quality or structural organization of heterochromatic elements may well alter the kind and/or degree of particular exchanges that occur, and in this way control the chromosome organization and the kind and the relative effectiveness of genic action. There can be little question that transpositions of both Ds and Ac occur and that the time of their occurrence in the development of a tissue is under precise control. This control is determined by the number of Ac loci present and their organization and possibly their position in the chromosome complement. Is this transposition of heterochromatin? Is it a reflection of a process that normally occurs in nuclei? Is it responsible for controlling the rates and types of exchange that occur between nucleus and cytoplasm? Is it usually an orderly mechanism, which is related to the control of the processes of differentiation? If so, induced disturbances in quantity and organization of the heterochromatic elements of the chromosome could give rise to a series of alterations reflected both in chromosome structure and behavior and in genic reactions that could

markedly alter phenotypic expressions.[10]   It is well known that the various knobs and centromeres may coalesce in the resting nuclei.   This coalescence is also frequently observed both in the somatic and the meiotic prophases. Are the transpositions and the changes in state of *Ac* products of this coalescence?   This is suspected because of the frequent transpositions of *Ac* from one chromosome to another.

It may be considered that these speculations with regard to heterochromatin behavior and function have been carried further than the evidence warrants.   This may be true; but it cannot be denied that one basic kind of phenomenon appears to underlie the expression of variegation in maize.   In many cases, there can be little question about the similarities in expression of variegation in Drosophila and maize.   A heterochromatic element has repeatedly been found to be basically associated with the origin and expression of variegation in Drosophila.   That a heterochromatic element likewise is responsible for the origin and behavior of variegation in maize has not been proved, although it is indicated, as the analysis of *Dt* has shown.

[1] Lewis, E. B., *Advances in Genetics*, **3**, 73–115 (1950).

[2] The symbols refer to genes affecting the parts of the plant as follows: *c*, aleurone pigment; *yg*, chlorophyll; *wx*, composition of starch in pollen and endosperm; *a*, aleurone pigment; *y*, starch composition of endosperm; *pyd*, chlorophyll.

[3] McClintock, B., Proc. Natl. Acad. Sci., **28**, 458–463 (1942).

[4] The annual reports of the author, appearing in the Yearbooks of the Carnegie Institution of Washington, **41–48**, (1942–1949), contain more detailed summaries of some of the observations that are described in this paper.

[5] Catcheside, D. G., *J. Genet.*, **38**, 345–352 (1939); *Ibid.*, **48**, 31–42 (1947); *Ibid.*, **48**, 99–110 (1947).

[6] Rhoades, M. M., *Genetics*, **23**, 377–397 (1938).   *Cold Spring Harbor Symposia Quant. Biol.*, **9**, 138–155 (1941); Proc. Natl. Acad. Sci., **31**, 91–95 (1945).

[7] Stern, C., *Genetics*, **21**, 625–730 (1936).

[8] McClintock, B., *Ibid.*, **26**, 234–282 (1941).

[9] Vanderlyn, L., *Bot. Rev.*, **15**, 507–582 (1949).

[10] This report deals only with the origin and behavior of mutable loci arising in these cultures.   A number of other heritable changes are also arising.   Many are associated with marked alterations in morphological characters.

GENERALIZED EISENSTEIN SERIES AND NON-ANALYTIC AUTOMORPHIC FUNCTIONS

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1. *Introduction.*—In a recent paper concerned with the uniqueness of Dirichlet series satisfying functional equations, Maass[4] introduced the concept of non-analytic automorphic functions. These are functions of two real variables, $f(x_1, x_2)$, possessing functional equations of the type

$$f(x_1, x_2 + c) = f(x_1, x_2),$$
$$f(x_1', x_2') = R(x_1, x_2) + s(x_1, x_2)f(x_1, x_2), \qquad (1)$$

where $x_1' + ix_2' = 1/(x_1 + ix_2)$.

These functions are connected with the zeta function of a quadratic number field, and their automorphic properties are deduced not as a direct consequence of the functional equations of the related Dirichlet series, nor as a consequence of the transformations of the associated theta functions, but, in a most interesting manner, as a result of the uniqueness of the solution of the partial differential equation satisfied by $f(x_1, x_2)$.

The purpose of this note is to indicate how a large class of non-analytic automorphic functions may be deduced from the fundamental analytic automorphic functions, the theta functions. Furthermore, the invariant character of these new functions will be demonstrated by deriving generalized Eisenstein series expansions for the functions. The associated Dirichlet series are the Epstein zeta functions.

Our basic tool is the class of functions, $W_a(x)$, defined by

$$W_a(x) = \int_0^\infty e^{-xv - 1/v} v^{-a} dv, \qquad Re(x) > 0, \qquad (2)$$

together with its generalizations and analogs, which we discuss below.

The case $a = \frac{3}{2}$ leads, with some non-essential changes, to the well-known formula,

$$e^{-\sqrt{z}} = \frac{1}{2\sqrt{\pi}} \int_0^\infty e^{-zv - 1/4v} v^{-1/2} dv, \qquad (3)$$

while the case $a = 1$ introduces a cylinder function, used by Maass in the construction of his functions.

As a first simple application of the method, we have

$$\sum_{m, n = -\infty}^{\infty} e^{-x\sqrt{\tau(m^2 + n^2)}} = \frac{1}{2\sqrt{\pi}} \int_{-\infty}^{\infty} \sum_{m, n = -\infty}^{\infty} e^{-\pi x^2(m^2 + n^2)v}$$
$$e^{-1/4v} v^{-1/2} dv. \qquad (4)$$

Applying the classical transformation formula, $\sum_{n=-\infty}^{\infty} e^{-n^2\pi t} = f(t) = t^{-1/2} f(1/t)$, this becomes

$$= \frac{1}{2\sqrt{\pi^{x^2}}} \int_{-\infty}^{\infty} \sum_{m,\,n=-\infty}^{\infty} e^{-\pi(m^2+n^2)/x^2 v} e^{-1/4v} v^{-1/2} dv, \qquad (5)$$

whence

$$\sum_{m,\,n=-\infty}^{\infty} e^{-\pi\sqrt{\pi(m^2+n^2)}} = 2x \sum_{m,\,n=-\infty}^{\infty} [4\pi(m^2+n^2) + x^2]^{-1/2}. \qquad (6)$$

This application of (3) is due to Hardy,[1] where many relations pertaining to the functions $W_a(x)$ are given. At the time this formula was first derived, as he himself noted subsequently, Hardy was not interested in any applications to number theory. When, afterwards,[2] he returned to[5](6) in connection with lattice points within a circle, he derived this formula in a different fashion. The possibilities of the method have not been fully explored, and we shall see that a slight extension of the method yields many interesting identities, identities which may be used to render obvious the automorphic behavior of the functions involved.

It will be clear from what follows that we may replace $m^2 + n^2$ by an arbitrary positive definite quadratic form in N variables. We will restrict ourselves to the simplest case in illustrating our method.

In place of the former series, we now consider

$$\sum_{m,\,n=-\infty}^{\infty} e^{-x_1\sqrt{\pi(m^2+n^2)} + 2imx_2 + 2inx_3} = \frac{1}{2\sqrt{\pi}} \int_{-\infty}^{\infty}$$

$$\sum_{m,\,n=-\infty}^{\infty} e^{-\pi x_1^2(m^2+n^2)v + 2imx_2 + 2inx_3} e^{-1/4v} v^{-1/2} dv. \qquad (7)$$

Inserting the transformation formula for $\sum e^{-\pi m^2 t_1 + 2imh}$ and carrying out the indicated integration with respect to v as before, we now obtain, after making the slight change, $x \to x\sqrt{4\pi}$,

$$\sum_{m,\,n=-\infty}^{\infty} e^{-2x_1\sqrt{\pi^2(m^2+n^2)} + 2imx_2 + 2inx_3} =$$

$$x_1(4\pi)^{-1/2} \sum_{m,\,n=-\infty}^{\infty} [(m+x_2)^2 + (n+x_3)^2 + x_1^2]^{-1/2}. \qquad (8)$$

If we now divide by x_1, take the partial derivatives of both sides of the equation with respect to x_1, divide again by x_1, transpose the term corresponding to $m = n = 0$ on the right-hand side to the left-hand side, set $x_1 = x_1'\sqrt{\mu^2 + v^2}$, $x_2 = \mu x_2'$, $x_3 = v x_3'$, and then sum over all values of μ and v apart from $(0, 0)$, we obtain on the right-hand side a constant multiple of the generalized Eisenstein series,

$$\sideset{}{'}\sum_{m, n, \mu, \nu} [(m + \mu x_2)^2 + (n + \nu x_3)^2 + (\mu^2 + \nu^2)x_1^2]^{-1/4} \quad (9)$$

(' indicates the $m = n = \mu = v = 0$ is excluded.) All these gyrations are due to the necessity of ensuring the convergence of the resulting expression, which does converge because the left-hand member converges.

Setting x_2 and x_3 equal, it is readily verified that we have a function satisfying (1) when $x_1 + ix_2 = 1/x_1' + ix_2'$.

2. In addition to the generalizations of the above result furnished by more general quadratic forms and the theta functions associated with algebraic number fields, an extensive generalization is obtained by replacing the class $W_a(x)$ of (2) above by the matrix functions

$$W_a(X) = \int e^{-tr(XV + V^{-1})} |V|^{-a} dV \quad (1)$$

where the integration is over the Siegel space of $n \times n$ positive definite matrices V, X is a positive definite matrix, and $|V|$ represents the determinant of V. The functions obtained by the analog of the previous method are real analogs of the generalized Eisenstein series of Siegel.[5] All this will be discussed in detail in a subsequent work in which we hope also to determine the connection between these new functions and the problem of Hecke considered by Maass.

3. As noted above, to obtain non-analytic automorphic functions of the type of Maass, we use the function $W_1(x)$. This function is the case $n = 2$ of the general Voronoi function, $V_n(x)$, defined by

$$\int_0^\infty V_n(x)x^{s-1}dx = \Gamma(s)^n. \quad (1)$$

This function was first introduced, I believe, by Voronoi in his classic memoir on the Dirichlet divisor problem. Using Mellin's inversion formula $V_n(x)$ may be represented by a complex integral. The analog of (2) of §1 also exists, namely,

$$V_n(x) = \int_0^\infty \cdots \int_0^\infty \frac{e^{-x t_1 t_2 \cdots t_n - 1/t_1 - 1/t_2 \cdots - 1/t_n}}{t_1 t_2 \cdots t_n} \pi dt_i. \quad (2)$$

From (1) it follows readily that the function defined by

$$f_k(x) = \sum_{n=1}^\infty d_k(n) V_k(n^2 x^2), \quad Re(x^2) > 0, \quad (3)$$

where $\zeta(s)^k = \sum_{n=1}^\infty d_k(n)n^{-s}$ possesses a functional equation connecting its value at x with its value at c_k/x, where c_k is a constant depending upon k. Furthermore, it may also be shown that

$$g_k(x) = \sum_{n=1}^\infty d_{2k}(n) V_k(n^2 x^2) \quad (4)$$

possesses an approximate functional equation, cf. reference 1, where this last result was used in connection with the mean value of $\zeta(s)$ on the critical line. The case $k = 1$ is due to Wigert.

It seems very difficult to establish corresponding results for

$$f_k(x, y) \;=\; \sum_{n=1}^{\infty} d_k(n) V_k(n^2 x^2) e^{iny} \tag{5}$$

[1] Bellman, R., "Wigert's Approximate Functional Equation and the Riemann Zeta-Function," *Duke Math. J.*, 16, 547–552 (1949).

[2] Hardy, G. H., "On Dirichlet's Divisor Problem," *Proc. Lond. Math. Soc.*, 15, 1–20 (1916).

[3] Hardy, G. H., "Some Multiple Integrals," *Quart. J. Math.*, 39, 357–375 (1908).

[4] Maass, H., "Uber eine neue Art von nichtanalytischen automorphen Funktionen und die Bestimmung Dirichetschen Reihen durch Funktionalgleichungen," *Math. Ann.*, 121, 141–183 (1949).

[5] Siegel, C. L., "Uber die analytische Theorie der quadratischen Formen," *Ann. Math.*, 136, 527–606 (1935).

ARITHMETICAL PROPERTIES OF POLYNOMIALS ASSOCIATED WITH THE LEMNISCATE ELLIPTIC FUNCTIONS

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1. I have studied elsewhere the arithmetical properties of certain polynomials associated with the real multiplication of elliptic functions.[1] Such polynomials include as a special case the function $U_n = (a^b - b)_n/(a - b)$ first systematically studied by Lucas[2] and Sylvester[3] when expressed as a polynomial in $P = a + b$ and $Q = ab$.

I have recently investigated the polynomials associated with the simplest type of complex multiplication of elliptic functions; namely, the so-called lemniscate case for which the period ratio τ has the value $i = \sqrt{-1}$ and the Weierstrass invariant g_3 is zero.

In the account which follows, the small greek letters α, ϵ, λ, μ, ν and τ will be used for elements of the ring G of Gaussian integers. $\bar{\alpha}$ and $N\alpha$ denote the conjugate and norm of α in G. α is said to be odd, oddly even or totally even according as $N\alpha$ is congruent to one, two or zero modulo 4. The letter ϵ is reserved for denoting any one of the four units ± 1, $\pm i$ of the ring G.

2. Let u be a complex variable, and $\wp(u)$ the Weierstrass $\wp$-function formed with the invariants $g_2 = 4w$, $g_3 = 0$. Let $E\mu = E\mu(u)$ equal 1,

$\sqrt{\wp(u)}$ or $\wp'(u)$ according as μ is odd, oddly even or totally even. Finally let

$$\Psi_\mu = \Psi_\mu(u) = \sigma(\mu u)/\sigma(u)^{N_\mu} \tag{1}$$

where $\sigma(u)$ is the Weierstrass sigma function. Then $\Psi_\mu + E_\mu$ is an even elliptic function with the same periods as $\wp(u)$. More specifically,

$$\Psi_\mu(u) = E_\mu(u)P_\mu(z, w) \tag{2}$$

where

$$P_\mu = P_\mu(z, w) = \sum_{r=0}^{q} \pi_r z^{q - 2r} w^r \tag{3}$$

is a polynomial in $z = \wp(u)$ and $w = \dfrac{g_2}{4}$ whose coefficients π_r are Gaussian integers with $\pi_0 = \mu$. The degree q of P_μ in z depends in a simple way on $N\mu$. The arithmetical properties of these polynomials were the object of the investigation; (3) is the elliptic function analog of the cyclotomic polynomial $\dfrac{z^n - 1}{z - 1}$ associated with Lucas' U_n.

3. The arithmetical properties of the polynomials P_μ closely parallel the properties of Lucas' U_n. The main new feature of interest (not occurring in the real multiplication case) is a genuine double numerical periodicity when the free variables z and w are given fixed values in G, and the residues of the resulting sequence in G are considered for moduli in G. Indeed Lucas claimed in his fundamental paper and elsewhere to have discovered doubly periodic numerical functions connected with the elliptic functions, but he apparently published nothing on this subject.[4]

The Lucas polynomial U_n may be defined as the solution of a simple difference equation with prescribed initial values. The function Ψ_μ may be similarly defined as a solution of the difference equation

$$\Omega_{\mu + \nu}\Omega_{\mu - \nu} = \Omega_{\mu + 1}\Omega_{\mu - 1}\Omega_\nu^2 - \Omega_{\nu + 1}\Omega_{\nu - 1}\Omega_\mu^2 \tag{4}$$

with prescribed initial values; in particular, $\Psi_0 = 0$ and $\Psi_1 = 1$. (A table of the corresponding initial values of P_μ for small $N\mu$ is given at the close of the paper.)

Consequently, just as in the real multiplication case,[5] the polynomials P_μ may be defined purely algebraically as modified solutions of (4). On using this algebraic definition in conjunction with the function-theoretic definitions (1) and (2), the following results were obtained.

(i) *If z, w are indeterminates, the correspondence $\nu \to P_\mu(z, w)$ is a mapping of the ring G into the polynomial ring $G(z, w)$ which preserves*

division; that is ν divides μ in G implies that P_ν divides P_μ in $G(z, w)$. Furthermore,

$$P_\epsilon = \epsilon, \qquad P_{\epsilon\mu} = \epsilon P_\mu, \qquad P\bar\mu = \bar P_\mu.$$

Therefore if μ is a rational integer, all the coefficients π_r of P_μ are rational integers, and P_μ reduces to the polynomial of the real multiplication case studied in reference 1.

Let z_0, w_0 be fixed rational integers. Then $h_\nu = P_\nu(z_0, w_0)$ is a Gaussian integer and the correspondence $\nu \to h_\nu$ is a mapping of G into itself preserving division. Let π from now on denote a fixed Gaussian prime. An integer λ is called a zero of h_ν modulo π if $h_\lambda \equiv 0(\text{mod } \pi)$ and a rank of apparition of π in $\{h_\nu\}$ if $h_\lambda \equiv 0(\text{mod } \pi)$ but $h_\mu \not\equiv 0(\text{mod }\pi)$ for μ any proper divisor of λ.

(ii) *If π is odd, the zeros of the prime π in $\{h_\nu\}$ form an ideal m which is never the zero ideal.* Furthermore if λ is any rank of apparition of π in $\{h_\nu\}$, m is the principal ideal determined by λ.

(iii) *If π is an odd complex Gaussian prime, then*[4]

$$P_\mu(z, w) \equiv P_\mu(0, w) \quad (\text{mod } \pi).$$

(iv) *The sequence $\{h_\nu\}$ becomes numerically periodic modulo π. The moduli of its periods is contained in the ideal m of its zeros modulo π.*

(v) *Given a specific term h_λ of $\{h_\nu\}$, the only odd primes π which can have rank of apparition λ in $\{h_\nu\}$ are either divisors of λ, or primes for which the polynomial $P_\lambda(z, w)$ splits completely into linear factors or completely into quadratic factors in the residue class ring $G(z, w)/(\pi)$.* Such primes lie in arithmetical progressions whose common constant difference is a function of λ alone.[7]

(v) generalizes the well-known result of Lucas and Sylvester that if P and Q are rational integers, all primitive prime divisors of U_l are either divisors of l or of the form $kl \pm 1$.

The first few polynomials P_μ are as follows: $P_0 = 0$, $P_1 = 1$, $P_i = i$, $P_{1+i} = 1 + i$, $P_2 = 2$, $P_3 = 3z^4 - 6wz^2 - w^2$, $P_{1+2i} = (1 + 2i)z^2 - w$, $P_{3+i} = (3 + i)z^4 - 2(1 + 3i)wz^2 + (3 + i)w^2$. All the remaining P_μ can be calculated from the recursion (4) and the relations $P_{\bar\mu} = \bar P_\mu$, $P_{\epsilon\mu} = \epsilon P_\mu$.

Qualitatively similar results hold for the polynomials associated with any complex multiplication of $\wp(u)$.[8]

A more complete account of these and other results with proofs will be published elsewhere.

[1] *Am. J. Math.*, 70, 31–74 (1948). Various algebraic properties of these polynomials are developed in Halphen's treatise on elliptic functions.

[2] *Ibid.*, 1, 184–240, 289–321 (1878).

[3] *Ibid.*, 2, 357–380 (1879).

⁴ In particular, Lucas stated to C. A. Laisant that there was a remarkable connection between his doubly periodic numerical functions and Fermat's last theorem. See Bell, E. T., *Bull. Am. Math. Soc.*, **29**, 401–406 (1923). The crux of the matter is to understand what Lucas meant by "double periodicity." Since the modules of the ring of integers are all principal ideals, no numerical function of the rational integer n can be doubly periodic. The simplest case in which double periodicity in the usually understood sense can occur is for numerical functions over the ring of Gaussian integers.

⁵ See Chapter V of reference 1.

⁶ Due to Eisenstein for the Jacobian lemniscate polynomials and used by him to prove the biquadratic reciprocity law. See his *Math. Abb.*, third paper or *J. Math. (Crelle)*, **30**, 184–187 (1846).

⁷ This result follows from Abel's theorem that the Galois group of the equation $P_\mu(z, w) = 0$ in z is commutative and of order q.

⁸ The equi-harmonic case when the period ratio τ is a complex cube root of unity and the invariant g_2 vanishes is being studied in detail by Lincoln K. Durst.

A NEW TYPE OF SELF-STERILITY IN PLANTS

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There are two well-known genetical systems controlling self-sterility in plants that is caused by incompatibility between a plant's own pollen and its pistil. One of these systems is associated with the phenomenon of heterostyly as reported in *Lythrum, Linum, Primula*, etc., by Darwin[1] and others. Lewis,[2] in his recent review, shows that the system found in heterostyled plants is characterized as follows: (1) One or two genes with two alleles; (2) coordinated gene action in pistil and pollen; (3) diploid (sporophytic) pollen control; (4) dominance.

In contrast to heterostyled plants, most homostyled plants, for example, *Nicotiana*, have the following system: (1) One gene with multiple alleles; (2) independent gene action in the pistil; (3) haploid (gametophytic) pollen control; (4) no dominance.

One previously reported exception among homostyled plants is found in *Capsella grandiflora* which possesses the system of heterostyled plants.

The present paper reports the discovery in two members of the Compositae of an entirely new system which is a combination of the first two features of homostyled plants and the last two features of heterostyled plants, as follows: (1) One gene with multiple alleles; (2) independent gene action in the pistil; (3) diploid (sporophytic) pollen control; (4) dominance in the anthers.

That some such new system exists in *Crepis* was first suspected by Babcock and Cave[3] in 1938. Their preliminary studies indicated that in *Crepis foetida* the genetical basis of self-sterility probably did not conform to either of the previously known schemes. The following year the junior author of the present paper undertook research on self-sterility in one subspecies of *Crepis foetida*. Although his doctoral thesis[4] was accepted in 1943, his report[5] is not yet published. It will appear, as a joint publication with the senior author of the present paper, in the September number of

Genetics. Meanwhile, Gerstel[6] has discovered that the *Crepis* system also exists in the well-known rubber plant, Guayule (*Parthenium argentatum*). His report of this discovery will appear in the July number of *Genetics.*

The genetical system controlling self-sterility in *Crepis* and *Parthenium* is unique in that it is a combination of certain of the features characterizing each of the two previously known systems. The theoretical possibility of the existence of such a system for the control of self-sterility was recognized by Lewis[2] in 1944, but its existence was then considered by him as hardly to be imagined.

The only serious difficulty concerns the second and fourth features of the new system: independent gene action in the pistil and dominance of certain alleles in the anthers. It was noted by Lewis,[2] however, that the immunity type of reaction involved in the physiological concept of the oppositional relation between alleles for self-sterility provides for the variation necessary to meet the requirements of a large multiple allele series together with the specificity necessary to provide for independent gene action in the pistil. Since the specificity of proteins is practically unlimited, is it not conceivable that in the Compositae, the most highly evolved of all flowering plants, protein relations have developed which provide just the types of specificity required for the independent action of alleles in the pistil and for diploid pollen control combined with dominance of certain alleles in the anthers?

In *Crepis* a plant's own pollen fails to germinate on the stigmatic surface. This reaction is obviously between two cytoplasms, both of which are under the influence of the same gene, or (as Lewis[7] recently postulated) of two self-reproducing parts of a gene, between which no crossing over occurs. Lewis[7] also reports that radiation experiments show that the S-gene behaves toward X-rays as though it were two independent units, one determining pollen reaction, the other, style reaction.

This concept that the S-gene may behave as a "dual" entity deserves further investigation.

[1] Darwin, C., *The Different Forms of Flowers on Plants of the Same Species*, D. Appleton & Co., New York (1896).

[2] Lewis, D., *Nature*, 153, 575–582 (1944).

[3] Babcock, E. B., and Cave, M. S., *Z. Ind. Abs. Vererb.*, 75, 124–160 (1938).

[4] Hughes, M. B., Thesis in archives, Univ. Calif. (1943).

[5] Hughes, M. B., and Babcock, E. B., *Genetics*, 35, in press.

[6] Gerstel, D. U., *Ibid.*, 35, in press.

[7] Lewis, D., *Biol. Rev.*, 24, 472–496 (1949).

"REPEATS" AND THE MODERN THEORY OF THE GENE

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Bridges introduced the term "repeats" for those sections of the salivary gland chromosomes of Drosophila which seem to be completely identical with other sections, the minimum extent of a repeat being a single band. He assumed that a section had once been actually reduplicated and had been inserted into the normal structure of the chromosome as a repeat, tandem or otherwise. He went one step further by suggesting that such repeats might account for the origin of new genes, if the repeated "gene" would change its function in the new position. Because the origin of new genes is completely mysterious, and because phylogeny without some such process is hardly conceivable within the framework of the gene theory, many geneticists have been found willing to accept this suggestion. This is rather surprising because the assumption is irreconcilable with the basic facts and tenets of classical genetics. A gene is supposed to reproduce its kind except when it mutates. The mutant gene, i.e., a member of a pair of alleles, has an action different from that of the original gene, and the same is true for any number of mutational changes, i.e., multiple alleles, but always affecting the same kind of process which, by way of extrapolation, is assumed to be controlled also by the original gene. Genes have never been known to mutate at different occasions into different directions, only into different grades of one effect. There is only one case known (the alleles spineless and aristapedia in *Drosophila melanogaster*) in which this relation does not seem to hold. To assume that a "repeated" gene can develop into a completely new type of gene, amounts, against the background of the classical conception of the gene and the facts of genetics, to mysticism. If it is pointed out that the new position of the gene makes a new effect possible—a vague allusion to the position effect—this is again an assumption which contradicts all known facts. Position effect produces the phenotype of a mutant of an adjacent locus (where known) as dominant or recessive effect, or, in special cases, as a mosaic effect. No fact is known which would justify the assumption that a change of position could make a known locus act otherwise than by producing its typical mutant effect, which includes the effects of multiple alleles.

Recently a number of facts have come to light which have been accounted for by the assumption of "repeats." The general type of these facts is this: Two or more mutants are found which behave like multiple alleles. Both produce, if homozygous (recessives), a definite effect, similar

but slightly different for each. In a compound the same effect is produced, which points to the presence of three multiple alleles. But careful experimentation reveals crossing over between the mutants, all other interpretations being excluded. Thus they behave as different "genes," though acting as alleles (pseudo-alleles, Lewis), and the conclusion is reached that they originated as repeats of one pre-existing gene. A number of such cases have now been studied (Lewis, Laughnan, Green and Green, Raffel and Muller, Komai) and others are suggested (Dunn and Caspari). An analysis of the facts, especially those unearthed in the remarkable work of Lewis and the Greens is apt to show the difficulties which the classical theory of the gene has to face and the superiority of a more modern concept.

Because Green and Green's work has thus far gone furthest inasmuch as a set of three "repeated" loci was found, we may use it as the basis of discussion. The decisive points are these: Among many lozenge alleles, all of which affect the quantity of the eye pigment, the eye structure, and the absence of the female spermathecas, and all of which behave as a typical series of multiple alleles, three could be shown thus far to exhibit a small amount of crossing over (resulting in one normal chromosome and one with more than one lozenge locus.) Thus they behave like individual loci, in close proximity, but permitting cross-over breaks between them. Nevertheless they act as alleles. This is best realized if different combinations are compared with at least one allele in both chromosomes with those in which one, two, or three alleles are present only in one chromosome. In a standard case of Mendelian inheritance individuals $\frac{a + c}{+ b +}$, $\frac{a \; b \; +}{+ \; + \; c}$ or $\frac{a \; b \; c}{+ + +}$, etc., should all be normal. But here only the last one is normal, the others show the compound lozenge effect, i.e., a, b and c behave as pseudo-alleles. The assumed "repeats" thus break the elementary rules of genetics and Green and Green know of no way out but to assume that in the first two cases the b or c, if separated from its mates, produces a position effect, thus giving the idea of position effect a quite new and doubtful definition, which was possibly suggested by a superficial resemblance to the original Bar position effect.

In my opinion these facts fit simply and without any new assumption into the modern picture of the basic features of the chromosome at the "genic" level. A group of facts are known (see especially Demerec 43 and Goldschmidt 44, full review and discussion in Goldschmidt 49) which show that the real genetical units of the chromosome are sections of different size, containing a number of bands in the salivary chromosomes, the maximum of which is not yet known. These units are characterized by the fact that whatever happens within this section produces a mutant effect of the same kind and that all these effects behave as multiple alleles. If we take, e.g.,

the "yellow" section invisible changes of a smaller order than one band, so-called point mutations, produce the mutant yellow. Translocation, inversion or deficiency breaks within this section act like mutants (position effect), also producing yellow, and all the point mutants and position effects behave as a series of multiple alleles. One may conclude that the invisible changes, the point mutants, are therefore also rearrangements, but within a single band. Whether this conclusion is drawn or not, it becomes clear that the whole section acts in some respects as a unit. Whatever happens within it, produces the same effect, or one very similar. If we should try to account for these facts by means of the classical theory of the gene, the whole section should be the gene because all changes within it are allelomorphic. The individual bands and their invisible mutations would become subgenes. But the position effects would also be subgenes. In addition, crossing over within the section seems possible. This is very unsatisfactory and the conclusion is obvious that at this level the classical theory of the gene does not work (see my former papers loc. cit.).

If we return now to the work on "repeats" it is seen to fit very easily into the group of facts and the concept just reported. At a former occasion (loc. cit.) I mentioned a disagreement between Muller and Demerec in regard to which band in the salivary chromosome should be regarded as the yellow locus. (There are similar discrepancies for other loci; see Bridges and Brehme, 1944.) The facts just reviewed led to the conclusion that there is no reason why both these authors should not be right. An invisible mutant change (point mutation) in any band of the yellow section would produce yellow. There can be at least as many point mutants of the same kind (and allelic) within any such section as there are bands. The application of this conclusion to the lozenge case is obvious: The lozenge effect is localized in a section which contains at least three bands which can mutate as so-called point-mutations. (Additional position effect alleles are bound to be discovered.) All must have a lozenge effect, all must be allelic just as it is proved for the yellow and scute sections, etc. (see Demerec and Goldschmidt, loc. cit.). No "repeats" are needed and no position effects. All facts fall in line simply if we forget about the classical theory of the gene and look at the facts dispassionately. Actually the work on the so-called repeats is a new proof of the correctness of that part of the newer ideas concerning the basic elements of genetics, which has been discussed here.

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ABSENCE OF MUTAGENIC ACTION OF X-RAYED CYTOPLASM IN HABROBRACON

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The literature concerned with x-ray effects on cells includes few records of attempts to separate injury induced in chromosomes from that induced in cytoplasm. Vintemberger[1] working with frog eggs concluded that it is the nucleus of the cell which is sensitive to x-rays. "L'irradiation de la région nucléaire a donc les mêmes effets que l'irradiation de la cellule entière." Dose used was 115 r. Zirkle[2] found that injury to fern spores by α-particles can be induced by extra-nuclear irradiation alone if dose is sufficiently large but that it is much greater when the nucleus is treated Astaurov[3] obtained androgenetic males from x-rayed Bombyx eggs fertilized by untreated sperm. These males (from untreated chromosomes in treated cytoplasm) were normal and their production continued after doses completely lethal to the expected types of progeny, biparental males and females. Henshaw[4] found a direct correlation between the presence of a nucleus at time of irradiation and the manifestation of an effect, delay in cleavage. He worked with nucleated and non-nucleated fragments of Arbacia eggs. Petrova[5] compared results of exposure to α-particles of entire cells of the alga Zygnema with those obtained by the treatment of the cytoplasm alone. She found that the mean lethal dose of the former ("Kerntod") was to that of the latter ("Plasmatod") as 1 to 700. Types of response differed under the two conditions of treatment. Transmissible changes were induced only when the entire cell had been irradiated.

What appears to be a striking exception to the conclusions of these inves-

tigators is the behavior of Amphibian eggs x-rayed in the germinal vesicle stage. Duryee[1–4] found that high doses of x-rays caused fragmentation of egg chromosomes, loss of lateral loops and separation of synaptic pairs as well as injury to the nucleolus when the nucleus was irradiated in the cell. Nuclei did not react markedly when irradiated after removal from the cell. Intermediate degrees of nuclear damage were produced by exposure of non-irradiated nuclei to x-rayed cytoplasm or to alkalinized Ringer's solution. These injuries appeared immediately following "as little as 30,000 r." Some changes could be detected with "as little as 10,000 r." Duryee concludes that "These data are consistent with a hypothesis that x-ray damage to nuclear components is not primarily a direct effect but an indirect one, probably caused by chemical changes in the cytoplasm."[5]

A consistent factor in the work of these authors is the high dose required for the induction of effect through cytoplasmic change. Duryee's statement that injuries can be induced by doses as low as 10,000 r stands in marked contrast to the reports of the cytogeneticists, most of whom work with relatively low doses. In fact, detailed analysis of chromosome changes induced by 50 r has been made several times.

Exceptionally favorable material for a further study of this problem is provided by Habrobracon. Normally, unfertilized eggs of this wasp develop into haploid males which are, of course, gynogenetic, while fertilized eggs develop into diploid biparental females. By suitable treatment with x-rays the chromosomes of an unfertilized egg can be made non-functional so that after fertilization the treated egg will develop as a haploid androgenetic male with paternal chromosomes only. If untreated sperm are used the possible mutagenic effects of the x-rayed cytoplasm on the untreated chromosomes can be tested. As indicated below, the experiments fail to show any evidence of mutagenic action on the part of the irradiated cytoplasm. Cytoplasm can be so seriously damaged by very high doses of x-rays as to prevent development, even of androgenetic males. The few androgenetic males surviving as this degree of injury is approached afford no evidence for mutagenic effect. This injury to Habrobracon cytoplasm is, in the opinion of the author, of the same nature as that induced by high doses administered to the cytoplasm and described in the works of the authors cited above.

The experiments on Habrobracon were conducted as follows: unmated homozygous wild type females were x-rayed and then mated to untreated males with one or more traits recessive to wild type. Dose ranged from 1350 r to 54,075 r. Three types of progeny were produced, the expected wild type biparental females and gynogenetic males and the exceptional androgenetic males, readily identifiable by their recessive paternal traits. The androgenetic males were carefully inspected for visible mutations.

Due to the fact that these males are haploid, both dominant and recessive mutations would be apparent in them. The biparental females were set unmated and their haploid sons studied for visible mutations. These females, half-sisters of androgenetic males, would be heterozygous for any visible mutations induced in the x-rayed egg chromosomes since they had developed from one set of x-rayed and one set of untreated chromosomes. The irradiated eggs were fertilized at times varying from one to several hours after exposure.

During the course of the experiments 6714 wild-type females were x-rayed, 2414 F_1 females tested and 75,546 F_2 males counted in the search for visible mutations (table 1). The number of androgenetic males at any one dose is too small to be significantly compared with their half-sisters

TABLE 1

DOSE IN r UNITS	VISIBLE MUTATIONS HETEROZYGOUS ♀♀		ANDROGENETIC ♂♂
	♀♀ TESTED	%	
1,350	1/111	0.90	1
2,025	1/246	0.40	4
6,000	1/285	0.35	7
7,210	1/54	1.85	0
12,000	30/585	5.13	13
14,420	9/139	6.47	22
21,630	31/422	7.34	31
28,840	18/223	8.07	39
34,608	....	..	9
36,050	37/324	11.42	24
40,000	....	...	3
43,260	3/25	12.00	4
Miscellaneous	....	...	13
	132/2414 = 5.470%		170

2P = 0.0002 (obtained by exact method of treating contingency tables).

in respect to visible mutation rate. A comparison of totals, 0/170 or 0% mutation rate for androgenetic males and 132/2414 or 5.470% for their half-sisters, indicates that the difference is highly significant. The exact method of treating contingency tables was used and 2 P was found to be 0.0002.

Androgenetic males of Habrobracon owe their *origin* to injury of irradiated egg *chromosomes*, their *survival* to absence of injury in the x-rayed egg *cytoplasm*.[10, 11] If these were the only factors involved in the incidence of androgenetic males, however, they would increase with increase in dose until the limit for their production was reached, the number of fertilized eggs capable of developing into them. Maximum number of such eggs per female is about thirteen. Maximum number of androgenetic males per female is about 0.12. This is attained at the relatively low dose of about 15,000 r. At higher doses a factor which reduces androgenetic males begins

to take effect and they decrease in number until none appears at about 54,000 r.[12] They have the same dose limit for survival as do gynogenetic males and biparental females. The single factor which all these classes of progeny have in common is x-rayed cytoplasm. Cytoplasm can, therefore, be injured by x-rays. Nevertheless it has exhibited no mutagenic effect. Of androgenetic males studied in the present work, 123 or 72.3% developed in cytoplasm which had been treated with doses high enough to induce detectable cytoplasmic injury. In spite of this they were normal in appearance and all tested were found to be fully fertile.

A question might be raised concerning the relative sensitivity to x-rays of the mitotic stages of the nuclei under discussion. The irradiated nuclei taking part in the formation of biparental females were treated in the diffuse stage of the first meiotic prophase. Sperm chromosomes become diffuse very soon after entering the egg. The chromosomes do not differ strikingly, either in degree of condensation or in tension, therefore, at times of exposure to possible mutagenic influences.

All results obtained from the study of the responses of Habrobracon eggs to x-rays are consistent in their indication that two kinds of change may be induced in the cell: (1) chromosome alterations connected with the production of dominant and recessive lethal and visible mutations and (2) a lethal effect directly cytoplasmic. The former are induced directly in the chromosomes or by transitory effect on the cytoplasm and have no lower threshold. The dose limits above which there is no survival may vary greatly in different mitotic stages of the same kind of cell. The latter has a high threshold and could not be detected in any cell with sensitive chromosomes except by such a method as the one used in this study. In the Habrobracon egg this cytoplasmic injury is constant in respect to dose for its incidence and dose for complete lethal action, irrespective of stage of the chromosomes at time of treatment.[10-12]

It is possible that mutagenic changes in chromosomes are induced by temporary alteration of irradiated cytoplasm since the shortest period between exposure of the egg and fertilization in the present study was one hour. This allowed time for recovery of cytoplasm before fertilization. If, however, there is such temporary cytoplasmic injury, there remains the conclusion, unavoidable to the author, that this is quite distinct from that induced by high doses which has been described by Duryee and Petrova.

The kinds of change with which the cytogeneticist works, "point" mutations, inversions, translocations, deletions, could not be identified in Duryee's material. These changes may have been induced in nuclei x-rayed after removal from the cytoplasm. The fact that such nuclei do not appear to have reacted markedly does not rule out mutational effects. The significance of the changes observed by Duryee in the three sets of eggs, nucleus x-rayed, cytoplasm x-rayed and both x-rayed, can be deter-

mined with certainty only if progeny can be obtained from them or if detailed analysis of chromosome behavior in subsequent meiotic divisions can be made. No author has reported induction of visible mutations by means of irradiation of the cytoplasm alone.

Conclusion.—X-rays can induce permanent change in the cytoplasm of Habrobracon eggs. This change may have a lethal effect on the egg but has not induced visible mutations in untreated chromosomes.

[1] Vintemberger, P., *Compt. rend. soc. biol.*, **99**, 1968 (1928).

[2] Zirkle, R. E., *J. Cellular Comp. Physiol.*, **2**, 251 (1932).

[3] Astaurov, B. L., *Biol. Zhur.*, **6**, 3 (1936).

[4] Henshaw, P. S., *Am. J. Cancer*, **33**, 258 (1938).

[5] Petrova, J., *Beihefte Bot. Centralbl.*, **61**, 399 (1942).

[6] Duryee, W. R., *Biol. Bull.*, **77**, 326 (1939).

[7] Duryee, W. R., *Anat. Record*, **75**, 144 (1939).

[8] Duryee, W. R., *Biol. Bull.*, **93**, 206 (1947).

[9] Since this paper was written the students in the Genetics Seminar at Swarthmore College have obtained twenty-nine additional androgenetic males. All were normal in appearance. Dose used was 28,000 r.

[10] Whiting, Anna R., *Am. Naturalist*, **79**, 193 (1945).

[11] Whiting, Anna R., *Biol. Bull.*, **95**, 354 (1948).

[12] Whiting, Anna R., *Ibid.*, **97**, 210 (1949).

A NOTE ON THE EXCEPTIONAL JORDAN ALGEBRA

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An associative algebra $\mathfrak{A}$ over a field $\mathfrak{F}$ is a vector space over $\mathfrak{F}$ together with an associative bilinear operation xy. When the characteristic of $\mathfrak{F}$ is not two we can use the same vector space and define a new algebra $\mathfrak{A}^{(+)}$ relative to the operation $\frac{1}{2}(xy + yx)$. This algebra is a Jordan algebra. Any Jordan algebra $\mathfrak{J}$ is called a *special* Jordan algebra if $\mathfrak{J}$ is isomorphic to a Jordan subalgebra of some $\mathfrak{A}^{(+)}$.

In 1934 it was shown[1] that the Jordan algebra $\mathfrak{G}$ of all three-rowed Hermitian matrices with elements in the simple eight-dimensional Cayley algebra $\mathfrak{C}$ is *exceptional* in the limited sense that $\mathfrak{G}$ is not isomorphic to a subalgebra of a finite-dimensional $\mathfrak{A}^{(+)}$. In the present note we shall give a simpler proof of the fact that $\mathfrak{G}$ is not a special Jordan algebra and *shall*

delete the restriction that $\mathfrak{A}$ *be finite dimensional.* We shall assume that
$\mathfrak{G}$ is imbedded in an associative algebra $\mathfrak{A}$ and shall then obtain a contradiction by showing that the enveloping associative algebra of $\mathfrak{G}$ contains
a subalgebra isomorphic to the non-associative algebra $\mathfrak{C}$.

We shall begin with the following background material. Let the
product in $\mathfrak{C}$ be designated by $x \cdot y$ and the involution of $\mathfrak{C}$ by $x \to \bar{x}$,
and let u be the unity quantity of $\mathfrak{C}$. The algebra $\mathfrak{G}$ is the supplementary
sum $\mathfrak{G} = \mathfrak{G}_{11} + \mathfrak{G}_{22} + \mathfrak{G}_{33} + \mathfrak{G}_{12} + \mathfrak{G}_{13} + \mathfrak{G}_{23}$ of vector spaces $\mathfrak{G}_{ij}$ for
$i \leq j$ where $\mathfrak{G}_{ii} = e_i \mathfrak{F}$ for pairwise orthogonal idempotents e_i whose sum
is the unity quantity of $\mathfrak{G}$. The spaces $\mathfrak{G}_{12}, \mathfrak{G}_{13}, \mathfrak{G}_{23}$ are isomorphic in the
vector space sense to $\mathfrak{C}$ and we express this isomorphism by writing x_{ij} for
the general quantity of $\mathfrak{G}_{ij}$ for every $i < j$, where x is the general quantity
of $\mathfrak{C}$. Then u defines a quantity u_{ij} such that

$$u_{ij}^2 = e_i + e_j. \tag{1}$$

The properties of $\mathfrak{G}$ may now be expressed in terms of the operation xy of
$\mathfrak{A}$ and we have

$$e_i x_{ij} + x_{ij} e_i = e_j x_{ij} + x_{ij} e_j = x_{ij}, \; e_k x_{ij} + x_{ij} e_k = 0 \tag{2}$$

for $i < j$ and $k \neq i, j$. We also have[2] the properties

$$x_{12} y_{23} + y_{23} x_{12} = (x \cdot y)_{13}, \; x_{12} y_{13} + y_{13} x_{12} = (\bar{x} \cdot y)_{23}, \tag{3}$$
$$x_{13} y_{23} + y_{23} x_{13} = (x \cdot \bar{y})_{12}.$$

Let us now proceed to our proof.

We first see that $0 = e_k(e_k x_{ij} + x_{ij} e_k) = (e_k x_{ij} + x_{ij} e_k)e_k$. Since $e_k^2 = e_k$
we have $e_k x_{ij} = x_{ij} e_k$ and so $2 e_k x_{ij} = 0$,

$$e_k x_{ij} = x_{ij} e_k = 0. \tag{4}$$

Write $f = e_i + e_j$ so that f is an idempotent and $fx_{ij} + x_{ij}f = 2x_{ij}$. Then
$2fx_{ij} = fx_{ij} + fx_{ij}f, fx_{ij} = fx_{ij}f = x_{ij}f$ and so

$$x_{ij} = x_{ij}(e_i + e_j) = (e_i + e_j)x_{ij}. \tag{5}$$

We finally see that $e_i y_{ij} e_i = e_i(y_{ij} - e_j y_{ij}) = e_i y_{ij} - e_i y_{ij} = 0$ and so we
have

$$e_i y_{ij} e_i = e_j y_{ij} e_j = 0. \tag{6}$$

Define

$$x' = e_1 x_{13} u_{12}$$

for every x of $\mathfrak{C}$. Then $(x \cdot y)' = e_1(x \cdot y)_{13} u_{12} = e_1(x_{12} y_{23} + y_{23} x_{12})u_{12} =$
$e_1 x_{12} y_{23} u_{12}$ by (3) and (4). Use the first equation of (3) with $y = u$, $x \cdot y = x$
to obtain $x_{13} = x_{12} u_{23} + u_{23} x_{12}$ whence $(x \cdot y)' = e_1(x_{12} u_{23} + u_{23} x_{12})y_{23} u_{12} =$
$e_1 x_{12} u_{23} y_{23} u_{12}$. By the second equation of (3) we have $y_{23} = y_{13} u_{12} + u_{12} y_{13}$

and so $(x \cdot y)' = e_1 x_{12} u_{23} (y_{12} u_{13} + u_{12} y_{13}) u_{13}$. Now (4), (5) and (6) imply that

$$y_{12} u_{13} u_{13} = y_{12} u_{13} (e_1 + e_3) u_{13} = (\mathfrak{H}_{23} - u_{13} y_{13}) e_1 u_{13} = -u_{13} y_{13} e_1 u_{13} =$$

$$-u_{13} (e_1 + e_3) y_{13} e_1 y_{13} = -u_{13} e_1 y_{13} e_1 u_{13} = 0.$$

Also $u_{23} u_{13} y_{13} = u_{23} u_{13} (e_1 + e_3) y_{13} = u_{23} u_{13} e_1 y_{13} = (u_{13} - u_{13} u_{23}) e_1 y_{13} = u_{13} e_1 y_{13}$. But then $(x \cdot y)' = e_1 x_{12} u_{13} e_1 y_{12} u_{13} = x' y'$.

We have proved that the mapping $x \to x'$ is a homomorphism of $\mathfrak{C}$ onto the subalgebra $\mathfrak{C}'$ of $\mathfrak{A}$ consisting of all x'. The kernel $\mathfrak{H}$ of this homomorphism is not $\mathfrak{C}$ since otherwise every $x' = 0$ whereas $u' = e_1 u_{12}^2 = e_1 (e_1 + e_2) = e_1 \neq 0$. Since $\mathfrak{C}$ is simple $\mathfrak{H} = 0$, the homomorphism is an isomorphism. This is impossible since $\mathfrak{C}$ is not associative and $\mathfrak{C}'$ is associative.

[1] See the author's "On a Certain Algebra of Quantum Mechanics," *Ann. Math.*, **35**, 65–73 (1934).

[2] For these properties see Section 18 of the author's "A Structure Theory for Jordan Algebras," *Ibid.*, **48**, 546–567 (1947).

ON THE SINGULAR VALUES OF A PRODUCT OF COMPLETELY CONTINUOUS OPERATORS

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In this note I wish to present a theorem on the singular values of a product of completely continuous operators in Hilbert space. As an application, a simple proof of a recent result of S. H. Chang will be given. The singular values of an operator K are the positive square roots of the eigen-values of K^*K, where K^* is the adjoint of K.

We begin with a slight generalization of a theorem of Weyl.[1]

THEOREM 1. *If H is a positive, symmetric, completely continuous operator whose first n eigen-values*[2] *are $\lambda_1, \ldots, \lambda_n$, then*

$$det \, [(Hy_i, y_j)] \leq \lambda_1 \cdot \ldots \cdot \lambda_n \, det \, [(y_i, y_j)]$$

for any elements $y_1, \ldots, y_n$.

Here, det $[a_{ij}]$ denotes the determinant of the nth order matrix with elements a_{ij}. Weyl's elegant proof uses an appeal to the theory of n-tensors. A straightforward proof may be given by using the relation $(Hy_i, y_j) = \sum_k \lambda_k (y_i, x_k)(x_k, y_j)$, where the x_k form a *complete* ortho-normal set.

THEOREM 2. *If K is a completely continuous operator with singular values α_i, then $\det [(Ky_i, Ky_j)] \leq \alpha_1^2 \ldots \alpha_n^2 \det [(y_i, y_j)]$.*

This follows immediately from Theorem 1 if we set $H = K^*K$.

THEOREM 3. *Let A and B be completely continuous operators and let the singular values of A, B and AB be denoted by α_i, β_i, γ_i, respectively. If f is any function such that $f(e^x)$ is convex and increasing as a function of x, then for each n we have $\sum_{i=1}^{n} f(\gamma_i) \leq \sum_{i=1}^{n} f(\alpha_i \beta_i)$.*

Proof: Let $y_1, \ldots, y_n$ form an ortho-normal set with $(AB)^*ABy_i = \gamma_i y_i$. By Theorem 2, $\gamma_1^2 \cdot \ldots \cdot \gamma_n^2 = \det [(ABy_i, ABy_j)] \leq \alpha_1^2 \cdot \ldots \cdot \alpha_n^2 \det [(By_i, By_j)] \leq \alpha_1^2 \cdot \ldots \cdot \alpha_n^2 \beta_1^2 \cdot \ldots \cdot \beta_n^2$. The result now follows by an application of a theorem of Polya.[3]

The next theorem was proved by Chang[4] using methods of function theory.

THEOREM 4. *Suppose $K = K_1 \cdot \ldots \cdot K_m$, where each K_i is an operator of finite norm (integral operator with L_2 kernel), and let γ_i be the singular values of K. Then $\sum_i \gamma_i^{2/m}$ converges.*

Proof: The proof is by induction on m. The case $m = 1$ is an immediate consequence of the definition of an operator of finite norm. Suppose the theorem holds when K is a product of fewer than m operators. Let α_i be the singular values of K_1, and let β_i be the singular values of $K_2 \cdot \ldots \cdot K_m$. Using Theorem 3 and Holder's inequality, we have

$$\sum_i \gamma_i^{2/m} \leq \sum_i \alpha_i^{2/m} \beta_i^{2/m} \leq \left(\sum_i \alpha_i^2\right)^{1/m} \left(\sum_i \beta_i^{2/m-1}\right)^{m-1/m}$$

In conclusion we remark that by a theorem of Chang,[4] the convergence of $\sum_i \gamma_i^{2/m}$ implies the convergence of $\sum_i |\lambda_i|^{2/m}$, where λ_i are the eigenvalues of K.

[1] Weyl, H., "Inequalities Between the Two Kinds of Eigenvalues of a Linear Transformation," these PROCEEDINGS, 35, 408–411 (1949).

[2] The eigen-values and singular values will always be arranged in order of decreasing absolute value, with repetitions according to multiplicity.

[3] Polya, G., "Remark on Weyl's Note: Inequalities Between the Two Kinds of Eigenvalues of a Linear Transformation," these PROCEEDINGS, 36, 49–51 (1950).

[4] Chang, S. H., "On the Distribution of the Characteristic Values and Singular Values of Linear Integral Equations," *Trans. Am. Math. Soc.*, 67, 351–368 (1949).

SOME NEW FUNCTIONS OF INTEREST IN X-RAY CRYSTALLOGRAPHY*

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In an earlier contribution[1] the relations between a *fundamental set* of points and its *vector set* were discussed, and it was shown that there are several ways of deriving the fundamental set from its vector set. The theory was confined to a discussion of vector sets based on fundamental sets composed of discrete unit points.

The theory can be readily extended to sets of weighted points and to density maps. Let fundamental space be divided into "unit" volumes. In each volume there are a number of points (which may be zero). Consider the particular "unit" volumes surrounding points a and b. Let the number of points in a's volume be m, and the number of points in b's volume be n. There are mn vectors from the points in a's volume to the points in b's volume; and there are nm vectors from the points in b's volume to the points in a's volume. If these vectors are now transferred to the common origin in vector space, they become two centrosymmetrical sheaves of vectors. The number of points at the end of each sheaf is mn. If the "unit" volumes are now reduced to zero, the m points in the volume at a come to coincide with point a, and the n points in the volume at b come to coincide with point b. In the vector set, the mn points in the volume at ab come to coincide with vector point ab. Since a is always associated with m, and b with n, the designations a and b may be used not only to indicate the points a and b but also may be permitted to carry the connotation of the number of points coincident at a and b, namely the weights of points a and b.

Now suppose that one decomposes a vector set into identical polygons.[1] For definiteness, suppose that the fundamental set is a five-point set, $a + b + c + d + e$, figure 1 (A). The decomposition is represented by equating the matrix of the vector set to the five images of the pentagon $a + b + c + d + e$, as follows:

$$
\begin{array}{ccccc}
aa & ab & ac & ad & ae \\
ba & bb & bc & bd & be \\
ca & cb & cc & cd & ce \\
da & db & dc & dd & de \\
ea & eb & ec & ed & ee
\end{array}
=
\begin{array}{l}
a(a + b + c + d + e) \\
b(a + b + c + d + e) \\
c(a + b + c + d + e). \\
d(a + b + c + d + e) \\
e(a + b + c + d + e)
\end{array}
\qquad (1)
$$

This decomposition demonstrates that, in general, each of the polygons in the vector set has a different weight, the various weights being the coeffi-

cients of the polynomials on the right of (1). As a consequence, the several different solutions of a vector set appear differently weighted, as diagrammatically indicated in figure 1 (*B*). If the weighting in the fundamental set is taken as the numbers of electrons in point-atoms, then the weighting in the vector set is that of the weights of the point-atoms in the squared-crystal.[3] If the weighting in the fundamental set is taken as the electron density, then the corresponding weighting in the vector set maps out the Patterson function.

The connection between vector maps of discrete points and the Patterson map can be traced as follows: Divide fundamental space and vector space
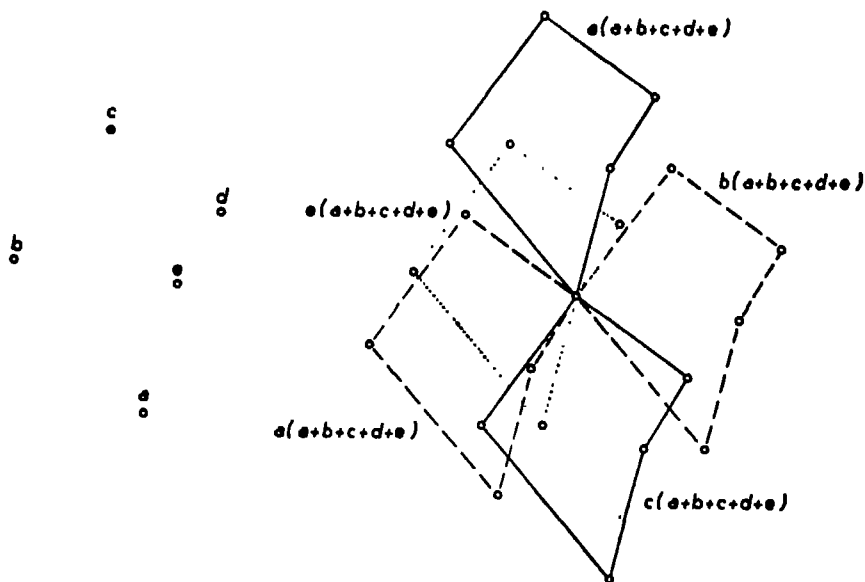
FIGURE 1A   FIGURE 1B

into elementary volumes, Δv. If the density of points at positions a and b in fundamental space are ρ_a and ρ_b, respectively, then there are $\rho_a \Delta v$ and $\rho_b \Delta v$ points in the elementary areas containing positions a and b. At the position ab in vector space there are $\rho_a \Delta v \times \rho_b \Delta v$ points in a volume $8\Delta v$. The density of points is therefore $\rho_a \Delta v \times \rho_b \Delta v / 8\Delta v = \frac{1}{8}\rho_a\rho_b \Delta v$. Neighboring cells of volume $8\Delta v$ overlap one another in such a way that each makes density contributions to neighboring cells. When this is taken into account, the actual density at point ab is $\rho_a\rho_b \Delta v$. As $\Delta v \to dv$, the value of the vector set weight at a particular point ab approaches $\rho_a\rho_b dv$. If there are other pairs of points in fundamental space parallel to $\overrightarrow{ab}$ which both have non-zero weights, then the weighting at ab in vector space is $\int_0^V \rho_a\rho_b dv$.

In the Patterson function, the average value of this product over the volume of the cell is chosen, namely $\dfrac{1}{V} \displaystyle\int_0^V \rho_a \rho_b dv$. Thus, the vector set of an electron density map has values at each point proportional to the Patterson function at these points.

For purposes to appear later, attention is next directed to the geometrical relation between the set of images of any particular polygon in the vector set. Let the vector set matrix be separated into images of a line, of a triangle, ... of an n-gon, as follows:

$$
\begin{matrix}
aa & ab & ac & ad & ae \\
ba & bb & bc & bd & be \\
ca & cb & cc & cd & ce \\
da & db & dc & dd & de \\
ea & eb & ec & ed & ee
\end{matrix}
\quad
\begin{matrix}
aa & ab & ac & a(d+e) \\
ba & bb & bc & b(d+e) \\
ca & cb & cc & c(d+e) \\
da & db & dc & d(d+e) \\
ea & eb & ed & e(d+e)
\end{matrix}
\quad
\begin{matrix}
aa & ab & a(c+d+e) \\
ba & bb & b(c+d+e) \\
ca & cb & c(c+d+e) \\
da & db & d(c+d+e) \\
ea & eb & e(c+d+e)
\end{matrix}
\quad (2)
$$

Consider the images indicated in the second part of (2). These can be written

$$
\begin{aligned}
a(d+e) &= ad + ae \\
b(d+e) &= bd + be \\
c(d+e) &= cd + ce. \\
d(d+e) &= dd + de \\
e(d+e) &= ed + ee
\end{aligned}
\qquad (3)
$$

The first column on the right side of (3) is simply the column polygon $(a + b + c + d + e)$ as imaged in d, and the second column is the same polygon imaged in e. In a similar manner, homologous points in a complete set of row images is a solution of the vector set which is centrosymmetrical with the solution which would have been achieved by completing any original row image. The centrosymmetrical aspect follows because corresponding row and column images are centrosymmetrical.

Sufficient groundwork is now established for considering a function which has the property of transforming a Patterson map into a representation of the electron density. If one could devise a function which would seek points located at one end of a line image, this collection of points would correspond to a column of the right of (3) and hence would be a weighted solution of the vector set. A function which has a high value when a line, whose components are xyz, spans two Patterson peaks is the product of the two Patterson functions at the ends of the line, namely

$$
\Pi_2(uvw, xyz) = P(uvw) \times P(u + x, v + y, w + z). \qquad (4)
$$

Such a function has maxima for values of uvw corresponding to all points which occur at the end of a line image of components xyz.

In using the function, it is undesirable to let x, y and z vary through their

entire range. If this were permitted, every point in the vector set would be reproduced by the function, for *every* vector point is related to other vector points by line images. Rather, it is desirable to set up the function to some particular line image. To accomplish this one first finds, in the Patterson map, a point image which is single. If this point has coordinates $x_1y_1z_1$, then the line from the origin to this point is one of a family of line images. The components of the line are $x_1y_1z_1$. If this specific value is placed in (4), then the function seeks the other points of the map which are at corresponding points of the line images of the rest of this family.
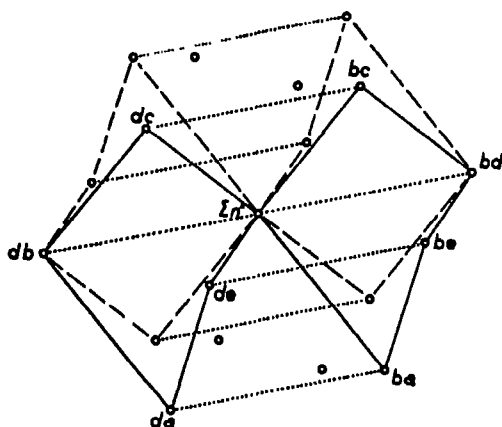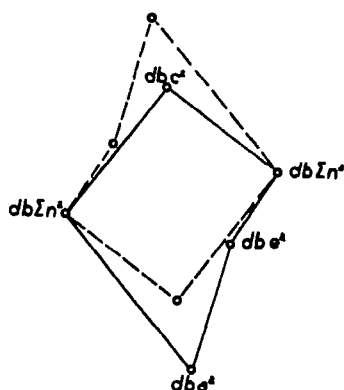
FIGURE 2A   FIGURE 2B

A more symmetrical form of this function may be arranged by requiring a high value of the function at a point at the center of gravity of the image. For line images, such a symmetrical function has the form

$$\Pi_2(uvw, xyz) = P\left(u - \frac{x}{2}, v - \frac{y}{2}, w - \frac{z}{2}\right) \times P\left(u + \frac{x}{2}, v + \frac{y}{2}, z + \frac{z}{2}\right). \quad (5)$$

Some of the characteristics of this function are illustrated in figure 2. Figure 2 (*A*) shows the vector set of figure 1 (*B*). Before the function is set up, one first makes the decision that some point in the vector set, namely *bd* in figure 2 (*A*), represents the image of a single point. The dotted line from the origin to this point is the line image which is thus established, and function (5) is then set up for the components of this line. The function has a value equal to the product of the values at the ends of this line as the line is shifted parallel to itself so as to range over the entire volume of the vector set. In the vector set of discrete points used for

illustration in figure 2 (A), the vector set has non-zero values only at the points shown by small circles. Wherever the image line spans a pair of points, the function lays down a peak at the center of the line. The map of the function and its values are shown in figure 2 (B). Note that the weight of a point in the function is the square of the weight in the fundamental set (Fig. 1 (A)) times a scale factor, db, with the proviso that points corresponding to images containing the origin are distorted to the origin weight of the vector set. The scale factor is the product used as the image point, namely bd. Thus, function (5) maps the electron density from Patterson F^2 data, except that it maps the density as its square, and that it maps the original image atom as having a weight equal to the Patterson origin weight.

Figure 2 (B) also shows that this map and its centrosymmetrical ghost appear together. When the function is applied to a centrosymmetrical crystal, a separate ghost can be eliminated if the origin point bd is chosen so that it represents a vector from an atom to its centrosymmetrical equivalent. In this case these enantiomorphous solutions coincide, and function (5) maps the desired atom positions in the crystal structure.

A proviso, tacitly assumed here, but explicitly discussed elsewhere,[*] is that if the original image point, bd, is not chosen at a single image, but corresponds to two coincident points derived from the ends of two different but parallel vectors in the fundamental set, then additional and spurious line images occur in figure 2 (A). As a consequence of this, figure 2 (B) contains more points than the crystal structure. In any case, it contains fewer points than the Patterson map. If the x-ray data are on a quantitative basis, it is currently possible to identify single image points. A convenient way to locate desirable image points is to use appropriate peaks in a Harker section.

Function (5) can be computed in either of two ways: If the numerical values are available for the Patterson function at an array of points uvw, which is usual after computing the function by using the Beevers-Lipson method[1] or some variation of it, then the Patterson function values separated by a line with components xyz can be multiplied to produce (5) at the midpoint of the line. This ordinarily requires 3600 products for a two-dimensional map unless the crystal has some symmetry, when this number is correspondingly less. Alternatively (5) can be recast into a Fourier synthesis based on the original F^2's. The form of the computation is found by substituting in (5) the values of the Patterson function, namely

$$P(uvw) = \frac{1}{V^2} \sum_h \sum_k \sum_l F^2_{hkl}\, e^{2\pi i(hu + kv + lw)}. \tag{6}$$

This gives

$$\Pi_2(uvw, xyz) = \frac{1}{V^2} \sum_h \sum_k \sum_l F^2_{h_1k_1l_1}\, e^{2\pi i(h_1[u - (x/2)] + k_1[v - (y/2)] + l_1[w - (z/2)])} \times$$

$$\frac{1}{V^2} \sum_{h_1} \sum_{h_1} \sum_{l_1} F^2_{h_1 h_1 l_1} \, e^{2\pi i ([h_1[u\,+\,(a/2)] \,+\, h_1[v\,+\,(v/2)] \,+\, l_1[w\,+\,(a/2)])} \quad (7)$$

$$= \frac{1}{V^4} \sum_{h_1} \sum_{h_2} \sum_{k_1} \sum_{k_2} \sum_{l_1} \sum_{l_2} F^2_{h_1 k_1 l_1} F^2_{h_2 k_2 l_2} \times$$

$$e^{2\pi i ([h_1\,-\,h_2]u/2 \,+\, [k_1\,-\,k_2]v/2 \,+\, [l_1\,-\,l_2]w/2)} \times$$

$$e^{2\pi i ([h_1\,+\,h_2]u \,+\, [k_1\,+\,k_2]v \,+\, [l_1\,+\,l_2]w)}. \quad (8)$$

This can be simplified for the usual experimental conditions, under which Friedel's law[4]

$$F^2_{hkl} = F^2_{\overline{hkl}} \quad (9)$$

holds. Under these conditions,

$$F^2_{h_1 k_1 l_1} F^2_{h_2 k_2 l_2} = F^2_{\overline{h_1 k_1 l_1}} F^2_{\overline{h_2 k_2 l_2}}, \text{ etc.,} \quad (10)$$

and the exponentials accompanying these coefficients in (8) determine opposite phases. In the entire summation, therefore, the imaginary components of the exponentials vanish, leaving only their real components, so that (8) can be rewritten

$$\Pi_3(uvw, xyz) = \frac{1}{V^4} \sum_{h_1} \sum_{h_2} \sum_{k_1} \sum_{k_2} \sum_{l_1} \sum_{l_2} F^2_{h_1 k_1 l_1} F^2_{h_2 k_2 l_2} \times$$

$$\cos 2\pi([h_1 - h_2]\frac{x}{2} + [k_1 - k_2]\frac{y}{2} + [l_1 - l_2]\frac{z}{2}) \times$$

$$\cos 2\pi([h_1 + h_2]u + [k_1 + k_2]v + [l_1 + l_2]w). \quad (11)$$

Part of the indicated summations as well as the first cosine term are not concerned with the Fourier computation proper, but merely relate to the formation of the compound Fourier coefficients. Thus (11) is a Fourier series resembling the standard electron density summation.

The decomposition of the vector set matrix into images shown in (2) indicates that homologous points of any complete set of polygon images maps out the fundamental set. Therefore, in a manner analogous to devising a function for finding line images, a function can be designed to find homologous points associated with any image. Suppose one has a Patterson map on which two peaks can be distinguished which (one has reason to believe) are both images of single points. Let their coordinates be $x_1 y_1 z_1$ and $x_2 y_2 z_2$. These two peaks and the origin constitute the image of a triangle with a point at the origin. For this triangle and all of the other images of the triangle, the function

$$\Pi_3(uvw, x_1 y_1 z_1, x_2 y_2 z_2) = P(uvw) \times P(u + x_1, v + y_1, w + z_1) \times$$
$$P(u + x_2, v + y_2, w + z_2) \quad (12)$$

has a high value. Therefore this function maps out the location of the atoms in the crystal structure. The value of this function at each point in the structure is the cube of the electron density, times a scale factor depending on the image points chosen, except that the density at the points corresponding to the origin triangles is exaggerated. (The form of (12), which corresponds with the form of (4) can also be expressed in a more symmetrical form corresponding with that of (5).)

In a similar manner more complicated image-seeking functions, Π_n, which seek images of a chosen n-gon, can be set up. The greater the value of n, the greater the distortion of the electron density mapped by the function, and the more tedious the computation, but the more certainly does the function reproduce variations in electron density rather than yield to fortuitous coincidences. This is because the function requires cooperation between n Patterson peaks to produce one electron density peak.

Since Π_2 maps the square of the electron density, Π_3 maps the cube of the electron density, etc., it follows that $\sqrt[2]{\Pi_2}, \sqrt[3]{\Pi_3}, \ldots \sqrt[n]{\Pi_n}$ map the electron density, except that atoms derived from images in the Patterson which contain the origin are exaggerated. It also follows that the real parts of the phases of the Fourier coefficients of the electron density function are the same as phases of the functions $\sqrt[2]{\Pi_2}, \sqrt[3]{\Pi_3} \ldots \sqrt[n]{\Pi_n}$ except that an allowance must be made for the exaggeration of the atoms arising from the origin image.

The discussion has been carried out largely in terms of three-dimensional Patterson functions. The successful use of Patterson functions for anything but simple structures depends largely on the use of three-dimensional functions, since two-dimensional and one-dimensional functions show bad overlapping of projected peaks.

The image-seeking function (11) has a resemblance to a function recently described by McLachlan.[5] The function given here differs in method of derivation and in the details of its form, specifically in the form of the Fourier coefficients.

* The subject matter of this paper formed part of a larger contribution entitled, "The Application of Image Theory to Crystal Structure Analysis," presented at the Phase and Computor Conference, Pennsylvania State College, April 6, 1950.

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Horsfall, Frank Lappin, Jr., 1948 (10), Hospital of the Rockefeller Institute for Medical Research, 66th Street and York Avenue, New York 21, N. Y.

Houston, William Vermillion, 1943 (3), The Rice Institute, Houston, Tex.

Hubble, Edwin Powell, 1927 (2), Mount Wilson Observatory, Pasadena, 4, Calif.

Hudson, Claude Silbert, 1927 (5), National Institutes of Health, Bethesda 14, Md.

Huggins, Charles Brenton, 1949 (10), Department of Surgery, University of Chicago, 950 East 59th Street, Chicago 37, Ill.

Hull, Albert Wallace, 1929 (3), Research Laboratory, General Electric Company, Schenectady 5, N. Y.

Hull, Clark Leonard, 1936 (12), Institute of Human Relations, Yale University, 333 Cedar Street, New Haven, Conn.

Hunsaker, Jerome Clark, 1935 (4), Massachusetts Institute of Technology, Cambridge 39, Mass.

Hunter, Walter Samuel, 1935 (12), Brown University, Providence 12, R. I.

Hutchinson, George Evelyn, 1950 (8), Osborn Zoological Laboratory, Yale University, New Haven 11, Conn.

Ipatieff, Vladimir Nikolaevich, 1939 (5), Universal Oil Products Company, Riverside, Ill.

Irwin, Malcolm Robert, 1950 (8), Department of Genetics, University of Wisconsin, Madison 6, Wis.

Ives, Herbert Eugene, 1933 (3), 32 Laurel Place, Upper Montclair, N. J.

Jacobs, Merkel Henry, 1939 (8), School of Medicine, University of Pennsylvania, Philadelphia 4, Pa.

Jacobs, Walter Abraham, 1932 (5), Rockefeller Institute for Medical Research, 66th Street and York Avenue, New York 21, N. Y.

Jeffries, Zay, 1939 (4), General Electric Company, 1 Plastics Avenue, Pittsfield, Mass.

Johnson, John Raven, 1948 (5), Department of Chemistry, Cornell University, Ithaca, N. Y.

Jones, Donald Forsha, 1939 (7), Box 1106, Department of Genetics, Connecticut Agricultural Experiment Station, New Haven 4, Conn.

Joy, Alfred Harrison, 1944 (2), Mount Wilson Observatory, Pasadena 4, Calif.

Kasner, Edward, 1917 (1), 430 West 116th Street, New York 27, N. Y.

Kelley, Walter Pearson, 1943 (6), 120 Hilgard Hall, University of California, Berkeley 4, Calif.

Kelly, Mervin J., 1945 (4), Bell Telephone Laboratories, 463 West Street, New York 14, N. Y.

Kelser, Raymond Alexander, 1948 (10), School of Veterinary Medicine, University of Pennsylvania, Philadelphia 4, Pa.

Kemble, Edwin Crawford, 1931 (3), Physics Laboratories, Harvard University, Cambridge 38, Mass.

Kendall, Edward Calvin, 1950 (9), Mayo Clinic, Rochester, Minn.

Kettering, Charles Franklin, 1928 (4), General Motors Corporation, Detroit 2, Mich.

Keyes, Frederick George, 1930 (5), Massachusetts Institute of Technology, Cambridge 39, Mass.

Kharasch, Morris Selig, 1946 (5), Department of Chemistry, University of Chicago, Chicago 37, Ill.

Kidder, Alfred Vincent, 1936 (11), 10 Frisbie Place, Cambridge 38, Mass.

King, Arthur Scott, 1941 (2), Mount Wilson Observatory, Pasadena 4, Calif.

Kirkwood, John Gamble, 1942 (5), Division of Chemistry, California Institute of Technology, Pasadena 4, Calif.

Kistiakowsky, George Bogdan, 1939 (5), Department of Chemistry, 12 Oxford Street, Harvard University, Cambridge 38, Mass.

Knopf, Adolph, 1931 (6), Yale University, New Haven, Conn.

Köhler, Wolfgang, 1947 (12), Swarthmore College, Swarthmore, Pa.

Kraus, Charles August, 1925 (5), Brown University, Providence 12, R. I.

Kroeber, Alfred L., 1928 (11), Department of Anthropology, Columbia University, New York 27, N. Y.

Kuiper, Gerard Peter, 1950 (2), Yerkes Observatory, University of Chicago, Williams Bay, Wis.

Kunkel, Louis Otto, 1932 (7), Rockefeller Institute for Medical Research, 66th Street and York Avenue, New York 21, N. Y.

Lamb, Arthur Becket, 1924 (5), Chemical Laboratory, Harvard University, Cambridge 38, Mass.

Lambert, Walter Davis, 1949 (2), P. O. Box 687, Canaan, Conn.

LaMer, Victor Kuhn, 1945 (5), Department of Chemistry, Columbia University, New York 27, N. Y.

Langmuir, Irving, 1918 (5), General Electric Company, Schenectady 5, N. Y.

Larsen, Esper S., Jr., 1944 (6), 2029 North Kentucky Street, Arlington, Va.

Lashley, Karl Spencer, 1930 (12), Yerkes Laboratories of Primate Biology, Orange Park, Fla.

Latimer, Wendell Mitchell, 1940 (5), University of California, Berkeley 4, Calif.

Lauritsen, Charles Christian, 1941 (3), California Institute of Technology, Pasadena 4, Calif.

Lawrence, Ernest Orlando, 1934 (3), Radiation Laboratory, University of California, Berkeley 4, Calif.

Lawson, Andrew Cowper, 1924 (6), University of California, Berkeley 4, Calif.

Lefschetz, Solomon, 1925 (1), Fine Hall, 129 Broadmead Street, Princeton, N. J.

Leith, Charles Kenneth, 1920 (6), Wardman Park Hotel, Washington 8, D. C.

Leuschner, Armin Otto, 1913 (2), 1816 Scenic Avenue, Berkeley 9, Calif.

Lewis, Howard Bishop, 1949 (9), Department of Biological Chemistry, Medical School, University of Michigan, Ann Arbor, Mich.

Lewis, Warren Harmon, 1936 (8), The Wistar Institute of Anatomy and Biology, Philadelphia 4, Pa.

Lewis, Warren Kendall, 1938 (4), Massachusetts Institute of Technology, Cambridge 39, Mass.

Libby, Willard Frank, 1950 (5), Institute for Nuclear Studies, University of Chicago, Chicago 37, Ill.

Lind, Samuel Colville, 1930 (5), P. O. Box P, Oak Ridge, Tenn.

Link, Karl Paul, 1946 (9), Department of Biochemistry, Agricultural Experiment Station, University of Wisconsin, Madison, Wis.

Linton, Ralph, 1945 (11), Institute of Human Relations, 333 Cedar Street, New Haven 11, Conn.

Lipmann, Fritz Albert, 1950 (9), Biochemical Research Laboratory, Massachusetts General Hospital, Boston 14, Mass.

Little, Clarence Cook, 1945 (10), Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Me.

Loeb, Leo, 1937 (10), 40 Crestwood Drive, St. Louis 5, Mo.

Loeb, Robert Frederick, 1946 (9), College of Physicians and Surgeons, 620 West 168th Street, New York 32, N. Y.

Long, Cyril Norman Hugh, 1948 (9), Yale University School of Medicine, 333 Cedar Street, New Haven 11, Conn.

Long, Esmond Ray, 1946 (10), Henry Phipps Institute, 7th and Lombard Streets, Philadelphia 47, Pa.

Longcope, Warfield Theobald, 1943 (10), Cornhill Farm, Lee, Mass.

Longsworth, Lewis Gibson, 1947 (5), Rockefeller Institute for Medical Research, 66th Street and York Avenue, New York 21, N. Y.

Longwell, Chester Ray, 1935 (6), Kirtland Hall, Yale University, New Haven, Conn.

Loomis, Alfred Lee, 1941 (4), The Loomis Laboratory, Room 2420, 14 Wall St., New York 5, N. Y.

Loomis, Francis Wheeler, 1949 (3), Department of Physics, University of Illinois, Urbana, Ill.

Lorente de Nó, Rafael, 1950 (9), Rockefeller Institute for Medical Research, 66th Street and York Avenue, New York 21, N. Y.

Lovering, Thomas Seward, 1949 (6), 4065 University Station, Tucson, Ariz.

Lowie, Robert Harry, 1931 (11), University of California, Berkeley 4, Calif.

Lyman, Theodore, 1917 (3), Research Laboratory of Physics, Harvard University, Cambridge 38, Mass.

McClintock, Barbara, 1944 (7), Carnegie Institution, Cold Spring Harbor, Long Island, N. Y.

McCollum, Elmer Verner, 1920 (9), Gilman Hall, Johns Hopkins University, Baltimore 18, Md.

McElvain, Samuel Marion, 1949 (5), Department of Chemistry, University of Wisconsin, Madison 6, Wis.

McMillan, Edwin Mattison, 1947 (3), Radiation Laboratory, University of California, Berkeley 4, Calif.

McShane, Edward James, 1948 (1), School of Mathematics, University of Virginia, Charlottesville, Va.

MacInnes, Duncan Arthur, 1937 (5), Rockefeller Institute for Medical Research, 66th Street and York Avenue, New York 21, N. Y.

Mac Lane, Saunders, 1949 (1), Department of Mathematics, University of Chicago, Chicago 37, Ill.

MacNider, William deBerniere, 1938 (9), University of North Carolina, Chapel Hill, N. C.

Macelwane, James Bernard, S.J., 1944 (6), 221 North Grand Boulevard, St. Louis 3, Mo.

Mangelsdorf, Paul Christoph, 1945 (7), Botanical Museum, Harvard University, Cambridge 38, Mass.

Mann, Frank Charles, 1950 (9), Mayo Foundation, University of Minnesota, Rochester, Minn.

Marshall, Eli Kennerly, Jr., 1943 (9), School of Medicine, Johns Hopkins University, 710 North Washington Street, Baltimore 5, Md.

Marvel, Carl Shipp, 1938 (5), Department of Chemistry, University of Illinois, Urbana, Ill.

Mason, Max, 1923 (3), 1035 Harvard Avenue, Claremont, Calif.

Maxcy, Kenneth Fuller, 1950 (10) School of Hygiene and Public Health, The Johns Hopkins University, 615 North Wolfe Street, Baltimore 5, Md.

Mayall, Nicholas Ulrich, 1949 (2), University of California, Lick Observatory, Mount Hamilton, Calif.

Mayer, Joseph Edward, 1946 (5), Department of Chemistry, University of Chicago, Chicago 37, Ill.

Maynard, Leonard Amby, 1944 (9), School of Nutrition, Cornell University, Ithaca, N. Y.

Mead, Warren Judson, 1939 (6), Massachusetts Institute of Technology, Cambridge 39, Mass.

Meek, Walter Joseph, 1947 (9), Department of Physiology, University of Wisconsin, Madison 6, Wis.

Mees, Charles Edward Kenneth, 1950 (5), Eastman Kodak Company, Kodak Park Works, Rochester 4, N. Y.

Mendenhall, Walter Curran, 1932 (6), 9 East Lenox Street, Chevy Chase 15, Md.

Menzel, Donald Howard, 1948 (2), Harvard College Observatory, Cambridge 38, Mass.

Merica, Paul Dyer, 1942 (4), 67 Wall Street, New York 5, N. Y.

Merrill, Elmer Drew, 1923 (7), Arnold Arboretum, Jamaica Plain, Mass.

Merrill, Paul Willard, 1929 (2), Mount Wilson Observatory, Pasadena 4, Calif.

Metz, Charles William, 1948 (8), University of Pennsylvania, Zoological Laboratory, 38th Street and Woodland Avenue, Philadelphia 4, Pa.

Meyer, Karl Friederich, 1940 (10), George Williams Hooper Foundation, University of California Medical Center, San Francisco 22, Calif.

Meyerhof, Otto, 1949 (9), Department of Physiological Chemistry, The School of Medicine, University of Pennsylvania, Philadelphia 4, Pa.

Miles, Walter Richard, 1933 (12), Yale University School of Medicine, 333 Cedar Street, New Haven 11, Conn.

Miller, George Abram, 1921 (1), 1203 West Illinois Street, Urbana, Ill.

Millikan, Robert Andrews, 1915 (3), California Institute of Technology, Pasadena 4, Calif.

Mitchell, Samuel Alfred, 1933 (2), Leander McCormick Observatory, University Station, Charlottesville, Va.

Moore, Carl Richard, 1944 (8), University of Chicago, Chicago 37, Ill.

Moore, Robert Lee, 1931 (1), University of Texas, Austin 12, Tex.

Morse, Harold Marston, 1932 (1), The Institute for Advanced Study, Princeton, N. J.

Moulton, Forest Ray, 1910 (2), 3027 N Street, N. W., Washington 7, D. C.

Mueller, John Howard, 1945 (10), Harvard Medical School, 25 Shattuck Street, Boston 15, Mass.

Muller, Hermann Joseph, 1931 (8), Science Hall 101, Indiana University, Bloomington, Ind.

Mulliken, Robert Sanderson, 1936 (3), University of Chicago, Chicago 37, Ill.

Murnaghan, Francis Dominic, 1942 (1), Johns Hopkins University, Baltimore 18, Md.

Murphree, Eger Vaughan, 1950 (4), Standard Oil Development Company, 15 West 51st Street, New York 19, N. Y.

Murphy, James Bumgardner, 1940 (10), Rockefeller Institute for Medical Research, 66th Street and York Avenue, New York 21, N. Y.

Nicholas, John Spangler, 1949 (8), Osborn Zoological Laboratory, Yale University, New Haven 11, Conn.

Nicholson, Seth Barnes, 1937 (2), Mount Wilson Observatory, Pasadena 4, Calif.

Nier, Alfred Otto C., 1950 (3), Department of Physics, University of Minnesota, Minneapolis 14, Minn.

Northrop, John Howard, 1934 (9), Department of Bacteriology, University of California, Berkeley 4, Calif.

Novy, Frederick George, 1924 (10), 721 Forest Avenue, Ann Arbor, Mich.

Noyes, William Albert, Jr., 1943 (5), Department of Chemistry, University of Rochester, Rochester 3, N. Y.

Oncley, John Lawrence, 1947 (9), University Laboratory of Physical Chemistry, Harvard University, 25 Shattuck St., Boston 15, Mass.

Onsager, Lars, 1947 (5), Sterling Chemistry Laboratory, Yale University, 225 Prospect Street, New Haven, Conn.

Opie, Eugene Lindsay, 1923 (10), Rockefeller Institute for Medical Research, 66th Street and York Avenue, New York 21, N. Y.

Oppenheimer, J. Robert, 1941 (3), The Institute for Advanced Study, Princeton, N. J.

Osterhout, Winthrop John Vanleuven, 1919 (7), Rockefeller Institute for Medical Research, 66th Street and York Avenue, New York 21, N. Y.

Painter, Theophilus Shickel, 1938 (8), University of Texas, Austin 12, Tex.

Palache, Charles, 1934 (6), Harvard University, Cambridge 38, Mass.

Parker, George Howard, 1913 (8), Harvard Biological Laboratories, Divinity Avenue, Cambridge 38, Mass.

Patterson, John Thomas, 1941 (8), Department of Zoology, University of Texas, Austin 12, Tex.

Paul, John Rodman, 1945 (10), Yale University School of Medicine, 333 Cedar Street, New Haven 11, Conn.

Pauling, Linus, 1933 (5), Crellin Laboratory, California Institute of Technology, Pasadena 4, Calif.

Pegram, George Braxton, 1949 (3), Columbia University, New York 27, N. Y.

Peters, John Punnett, 1947 (9), Department of Internal Medicine, Yale University School of Medicine, New Haven 11, Conn.

Pierce, George Washington, 1920 (3), Cruft Laboratory, Harvard University, Cambridge 38, Mass.

Piggot, Charles Snowden, 1946 (3), United States Embassy, London, England

Pillsbury, Walter Bowers, 1925 (12), University of Michigan, Ann Arbor, Mich.

Pitzer, Kenneth Sanborn, 1949 (5), Division of Research, U. S. Atomic Energy Commission, 1901 Constitution Avenue, Washington 25, D. C.

Rabi, Isidor Isaac, 1940 (3), Department of Physics, Columbia University, New York 27, N. Y.

Raper, Kenneth Bryan, 1949 (7), Northern Regional Research Laboratory, U. S. Department of Agriculture, Peoria 5, Ill.

Raymond, Arthur Emmons, 1950 (4), Douglas Aircraft Company, Inc., 3000 Ocean Park Boulevard, Santa Monica, Calif.

Reeside, John Bernard, Jr., 1945 (6), Box 177, Hyattsville, Md.

Reichelderfer, Francis Wilton, 1945 (3), Room 310, U. S. Weather Bureau, Washington 25, D. C.

Rhoades, Marcus Morton, 1946 (7), Department of Botany, University of Illinois, Urbana, Ill.

Richards, Alfred Newton, 1927 (9), School of Medicine, University of Pennsylvania, Philadelphia 4, Pa.

Richter, Curt Paul, 1948 (12), The Johns Hopkins Hospital, Baltimore 5, Md.

Riddle, Oscar, 1939 (8), Route 4, Plant City, Fla.

Ritt, Joseph Fels, 1933 (1), Columbia University, New York 27, N. Y.

Rivers, Thomas Milton, 1934 (10), Rockefeller Institute for Medical Research, 66th Street and York Avenue, New York 21, N. Y.

Robbins, William Jacob, 1940 (7), New York Botanical Garden, Bronx Park (Fordham Station), New York 58, N. Y.

Robertson, Oswald Hope, 1943 (10), Department of Biology, Stanford University, Stanford University, Calif.

Rodebush, Worth Huff, 1938 (5), University of Illinois, Urbana, Ill.

Romer, Alfred Sherwood, 1944 (8), Museum of Comparative Zoology, Oxford Street, Cambridge 38, Mass.

Rose, William Cumming, 1936 (9), University of Illinois, Urbana, Ill.

Ross, Frank Elmore, 1930 (2), Mount Wilson Observatory, Pasadena 4, Calif.

Rossby, Carl-Gustaf Arvid, 1943 (3), Institute of Meteorology, University of Chicago, Chicago 37, Ill.

Rossi, Bruno Benedetto, 1950 (3), Department of Physics, Massachusetts Institute of Technology, Cambridge 39, Mass.

Rous, Francis Peyton, 1927 (10), Rockefeller Institute for Medical Research, 66th Street and York Avenue, New York 21, N. Y.

Rubey, William Walden, 1945 (6), United States Geological Survey, Washington 25, D. C.

Ruedemann, Rudolf, 1928 (6), 161 Dana Avenue, Albany 3, N. Y.

Russell, Henry Norris, 1918 (2), Princeton University, Princeton, N. J.

Sabin, Florence Rena, 1925 (10), 1333 East 10th Avenue, Denver 3, Colo.

Saunders, Frederick Albert, 1925 (3), South Hadley, Mass.

Savage, John Lucian, 1949 (4), 1651 Dahlia Street, Denver 7, Colo.

Sax, Karl, 1941 (7), Harvard University, Cambridge 38, Mass.

Scatchard, George, 1946 (5), Department of Chemistry, Massachusetts Institute of Technology, Cambridge 39, Mass.

Schlesinger, Hermann Irving, 1948 (5), Department of Chemistry, University of Chicago, Chicago 37, Ill.

Schmidt, Carl Frederic, 1949 (9), Laboratory of Pharmacology, School of Medicine, University of Pennsylvania, Philadelphia 4, Pa.

Schmitt, Francis Otto, 1948 (8), Department of Biology, Massachusetts Institute of Technology, Cambridge 39, Mass.

Schultz, Adolph Hans, 1939 (11), Johns Hopkins Medical School, Baltimore 5, Md.

Schwinger, Julian, 1949 (3), Department of Physics, Harvard University, Cambridge 38, Mass.

Seaborg, Glenn Theodore, 1948 (5), Department of Chemistry, University of California, Berkeley 4, Calif.

Seares, Frederick Hanley, 1919 (2), 351 Palmetto Drive, Pasadena 2, Calif.

Shaffer, Philip Anderson, 1928 (9), Washington University Medical School, St. Louis 10, Mo.

Shapiro, Harry Lionel, 1949 (11), Department of Anthropology, American Museum of Natural History, Central Park West at 79th Street, New York 24, N. Y.

Shapley, Harlow, 1924 (2), Harvard College Observatory, Cambridge 38, Mass.

Sherman, Henry Clapp, 1933 (9), Columbia University, New York 27, N. Y.

Shope, Richard Edwin, 1940 (10), Box 323, Kingston, N. J.

Simpson, George Gaylord, 1941 (6), American Museum of Natural History, 77th Street and Central Park West, New York 24, N. Y.

Sinnott, Edmund Ware, 1936 (7), Osborn Botanical Laboratory, Yale University, New Haven, Conn.

Skinner, Burrhus Frederic, 1950 (12), Psychological Laboratories, Harvard University, Cambridge 38, Mass.

Slater, John Clarke, 1932 (3), Massachusetts Institute of Technology, Cambridge 39, Mass.

Slepian, Joseph, 1941 (4), Westinghouse Electric Corporation, East Pittsburgh, Pa.

Slichter, Louis Byrne, 1944 (3), 1446 North Amalfi Drive, Pacific Palisades, Calif.

Slipher, Vesto Melvin, 1921 (2), Lowell Observatory, Flagstaff, Ariz.

Small, Lyndon Frederick, 1941 (5), National Institutes of Health, Bethesda 14, Md.

Smith, Gilbert Morgan, 1948 (7), School of Biological Sciences, Stanford University, Stanford University, Calif.

Smith, Homer William, 1945 (9), 477 First Avenue, New York 16, N. Y.

Smith, Lee Irvin, 1944 (5), School of Chemistry, University of Minnesota, Minneapolis 14, Minn.

Smith, Paul Althaus, 1947 (1) Department of Mathematics, Columbia University, New York 27, N. Y.

Smith, Philip Edward, 1939 (8), College of Physicians and Surgeons, 630 West 168th Street, New York 32, N. Y.

Soderberg, Carl Richard, 1947 (4), Massachusetts Institute of Technology, Cambridge 39, Mass.

Sonneborn, Tracy Morton, 1946 (8), Department of Zoology, Indiana University, Bloomington, Ind.

Spier, Leslie, 1946 (11), P. O. Box 880, Santa Cruz, Calif.

Stadie, William Christopher, 1945 (9), 821 Maloney Clinic, 36th and Spruce Streets, Philadelphia 4, Pa.

Stadler, Lewis John, 1938 (7), University of Missouri, Columbia, Mo.

Stakman, Elvin Charles, 1934 (7), University Farm, St. Paul 8, Minn.

Stanley, Wendell Meredith, 1941 (9), Department of Biochemistry, University of California, Berkeley 4, Calif.

Stebbins, Joel, 1920 (2), Lick Observatory, Mt. Hamilton, Calif.

Stern, Curt, 1948 (8), Department of Zoology, University of California, Berkeley 4, Calif.

Stern, Otto, 1945 (3), 759 Cragmont Avenue, Berkeley, Calif.

Stevens, Stanley Smith, 1946 (12), Department of Psychology, Harvard University, Cambridge 38, Mass.

Stewart, George Walter, 1938 (3), State University of Iowa, Iowa City, Iowa

Stock, Chester, 1948 (6), Division of Geological Sciences, California Institute of Technology, Pasadena 4, Calif.

Stone, Calvin Perry, 1943 (12), Stanford University, Stanford University, Calif.

Stone, Marshall Harvey, 1938 (1), 313 Eckhart Hall, University of Chicago, Chicago 37, Ill.

Stratton, Julius Adams, 1950 (4), Massachusetts Institute of Technology, Cambridge 39, Mass.

Struve, Otto, 1937 (2), Department of Astronomy, University of California, Berkeley 4, Calif.

Sturtevant, Alfred Henry, 1930 (8), California Institute of Technology, Pasadena 4, Calif.

Suits, Chauncey Guy, 1946 (4), Research Laboratory, General Electric Company, Schenectady 5, N. Y.

Sumner, James Batcheller, 1948 (9), New York State College of Agriculture, Laboratory of Enzyme Chemistry, Cornell University, Ithaca, N. Y.

Sverdrup, Harald Ulrik, 1945 (3), Norsk Polarinstitutt, Observatoriegt. 1, Oslo, Norway

Swanton, John Reed, 1932 (11), 22 George Street, Newton 58, Mass.

Taliaferro, William Hay, 1940 (8), University of Chicago, Chicago 37, Ill.

Teller, Edward, 1948 (3), P. O. Box 1663, Los Alamos, N. Mex.

Terman, Frederick Emmons, 1946 (4), School of Engineering, Stanford University, Stanford University, Calif.

Terman, Lewis Madison, 1928 (12), 761 Dolores Street, Stanford University, Calif.

Thimann, Kenneth Vivian, 1948 (7), Harvard University, Biological Laboratories, 16 Divinity Avenue, Cambridge 38, Mass.

Thom, Charles, 1937 (7), 207 Grant Street, Port Jefferson, N. Y.

Thomas, Charles Allen, 1948 (4), Monsanto Chemical Company, 1700 South Second Street, St. Louis 4, Mo.

Thomas, Tracy Yerkes, 1941 (1), Swain Hall, Indiana University, Bloomington, Ind.

Thurstone, Louis Leon, 1938 (12), University of Chicago, Chicago 37, Ill.

Timoshenko, Stephen Prokop, 1940 (4), Room 262, Engineering Building, Stanford University, Calif.

Tolman, Edward Chace, 1937 (12), Department of Psychology, University of California, Berkeley 4, Calif.

Tozzer, Alfred Marston, 1942 (11), Peabody Museum, Harvard University, Cambridge 38, Mass.

Trumpler, Robert Julius, 1932 (2), Berkeley Astronomical Department, University of California, Berkeley 4, Calif.

Tuve, Merle Antony, 1946 (3), Department of Terrestrial Magnetism, Carnegie Institution of Washington, 5241 Broad Branch Road, N. W., Washington 15, D. C.

Twitty, Victor Chandler, 1950 (8), Department of Biological Sciences, Stanford University, Stanford, Calif.

Tyzzer, Ernest Edward, 1942 (10), 175 Water Street, Wakefield, Mass.

Urey, Harold Clayton, 1935 (5), Institute for Nuclear Studies, University of Chicago, Chicago 37, Ill.

Vandiver, Harry Shultz, 1934 (1), Department of Applied Mathematics, University of Texas, Austin 12, Tex.

Van Niel, Cornelis Bernardus, 1945 (7), Hopkins Marine Station of Stanford University, Pacific Grove, Calif.

Van Slyke, Donald Dexter, 1921 (9), Brookhaven National Laboratory, Upton, Long Island, N. Y.

Van Vleck, John Hasbrouck, 1935 (3), Harvard University, Cambridge 38, Mass.

Vaughan, Thomas Wayland, 1921 (6), 3333 P Street, N. W., Washington 7, D. C.

Veblen, Oswald, 1919 (1), The Institute for Advanced Study, Princeton, N. J.

Vickery, Hubert Bradford, 1943 (9), Connecticut Agricultural Experiment Station, New Haven 4, Conn.

von Karman, Theodore, 1938 (4), 1501 South Marengo Avenue, Pasadena, Calif.

von Neumann, John, 1937 (1), The Institute for Advanced Study, Princeton, N. J.

Waksman, Selman Abraham, 1942 (7), Agricultural Experiment Station, New Brunswick, N. J.

Wald, George, 1950 (9), Biological Laboratories, Harvard University, 16 Divinity Avenue, Cambridge 38, Mass.

Walker, John Charles, 1945 (7), 206 Horticulture Building, University of Wisconsin, Madison 6, Wis.

Walsh, Joseph Leonard, 1936 (1), Harvard University, Cambridge 38, Mass.

Webster, David Locke, 1923 (3), Physics Department, Room 385, Stanford University, Stanford University, Calif.

Weiss, Paul Alfred, 1947 (8), Department of Zoology, University of Chicago, Chicago 37, Ill.

Went, Frits Warmolt, 1947 (7), California Institute of Technology, Pasadena 4, Calif.

Werkman, Chester Hamlin, 1946 (9), Department of Bacteriology, Iowa State College, Ames, Iowa

Wetmore, Alexander, 1945 (8), Smithsonian Institution, Washington 25, D. C.

Wever, Ernest Glen, 1940 (12), Princeton University, Princeton, N. J.

Weyl, Claus Hugo Hermann, 1940 (1), The Institute for Advanced Study, Princeton, N. J.

Whipple, George Hoyt, 1929 (10), School of Medicine and Dentistry, University of Rochester, 260 Crittenden Boulevard, Rochester 7, N. Y.

Whitehead, John Boswell, 1932 (4), Johns Hopkins University, Baltimore 18, Md.

Whitney, Hassler, 1945 (1), Eliot House N21, Cambridge 38, Mass.

Wigner, Eugene Paul, 1945 (3), 8 Ober Road, Princeton, N. J.

Williams, Howel, 1950 (6), Department of Geological Sciences, University of California, Berkeley 4, Calif.

Williams, Robert R., 1945 (5), 297 Summit Avenue, Summit, N. J.

Williams, Roger John, 1946 (5), Biochemical Institute, University of Texas, Austin 12, Tex.

Willier, Benjamin Harrison, 1945 (8), Department of Biology, Johns Hopkins University, Baltimore 18, Md.

Wilson, Edgar Bright, Jr., 1947 (5), Harvard University, Department of Chemistry, 12 Oxford Street, Cambridge 38, Mass.

Wilson, Edwin Bidwell, 1919 (3), Harvard School of Public Health, 695 Huntington Avenue, Boston 15, Mass.

Wilson, Ralph Elmer, 1950 (2), Mt. Wilson and Palomar Observatories, 813 Santa Barbara Street, Pasadena 4, Calif.

Wilson, Robert Erastus, 1947 (4), 910 South Michigan Avenue, Chicago 80, Ill.

Wintersteiner, Oskar, 1950 (9), Squibb Institute for Medical Research, New Brunswick, N. J.

Wislocki, George Bernays, 1941 (8), Harvard Medical School, 25 Shattuck Street, Boston 15, Mass.

Wolbach, Simeon Burt, 1938 (10), Department of Pathology, Children's Hospital, 300 Longwood Avenue, Boston 15, Mass.

Wolfrom, Melville Lawrence, 1950 (9), Department of Chemistry, Ohio State University, Columbus 10, Ohio

Wood, Robert Williams, 1912 (3), Johns Hopkins University, Baltimore 18, Md.

Woodring, Wendell Phillips, 1946 (6), United States Geological Survey, Washington 25, D. C.

Woodworth, Robert Sessions, 1921 (12), Columbia University, New York 27, N. Y.

Wright, Frederick Eugene, 1923 (6), 2134 Wyoming Avenue, N. W., Washington 8, D. C.

Wright, Sewall Green, 1934 (8), Department of Zoology, University of Chicago, Chicago 37, Ill.

Wright, William Hammond, 1922 (2), 60 North Keeble Avenue, San Jose 11, Calif.

Wulf, Oliver Reynolds, 1949 (5), Weather Bureau Regional Office, Gates and Crellin Laboratories, California Institute of Technology, Pasadena 4, Calif.

Wyckoff, Ralph Walter Graystone, 1949 (5), Laboratory of Physical Biology, National Institutes of Health, Bethesda 14, Md.

Yerkes, Robert Mearns, 1923 (12), Yale University School of Medicine, 333 Cedar Street, New Haven, Conn.

Yost, Don Merlin Lee, 1944 (5), California Institute of Technology, Pasadena 4, Calif.

Zachariasen, Frederik William Houlder, 1949 (3), Department of Physics, University of Chicago, Chicago 37, Ill.

Zariski, Oscar, 1944 (1), Department of Mathematics, Harvard University, Cambridge 38, Mass.

Zworykin, Vladimir Kosma, 1943 (4), 103 Battle Road, Princeton, N. J.

Number of Members July 1, 1950:   461

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Allen, Charles Elmer, 1924, Biology Building, University of Wisconsin, Madison 6, Wis.

Anderson, John August, 1928, P. O. Box 332, Pasadena 17, Calif.

Benedict, Francis Gano, 1914, Machiasport, Me.

Child, Charles Manning, 1935, Jordan Hall, Stanford University, Stanford University, Calif.

Dewey, John, 1910, 1158 Fifth Avenue, New York 29, N. Y.

Dickson, Leonard Eugene, 1913, Route #2, Joliet, Ill.

Fernald, Merritt Lyndon, 1935, Gray Herbarium, Harvard University, Cambridge 38, Mass.

Hektoen, Ludvig, 1918, Chicago Tumor Institute, 21 West Elm Street, Chicago 10, Ill.

Hulett, George Augustus, 1922, 44 Washington Road, Princeton, N. J.

Stratton, George Malcolm, 1928, University of California, Berkeley 4, Calif.

Whitney, Willis Rodney, 1917, General Electric Company, Schenectady 5, N. Y.

FOREIGN ASSOCIATES

The letter in parentheses following the year of election indicates the field of scientific research in which the foreign associate was working at the time of his election, as follows:

(A)	Mathematics ·	(G) Botany
(B)	Astronomy	(H) Zoology and Anatomy
(C)	Physics	(I) Physiology and Biochemistry
(D)	Engineering	(J) Pathology and Bacteriology
(E)	Chemistry	(K) Anthropology and Psychology
(F)	Geology and Paleontology	

Adrian, Edgar Douglas, 1941 (I), Trinity College, Cambridge, England

Alexandroff, Paul A., 1947 (A), Mathematical Institute of the Academy of Sciences of the U.S.S.R., Bolshaya Kalushskaya 19, Moscow, U.S.S.R.

Bailey, Sir Edward, 1944 (F), 19 Greenhill Gardens, Edinburgh 10, Scotland

Bartlett, Sir Frederic Charles, 1947 (K), University of Cambridge, The Psychological Laboratory, Downing Place, Cambridge, England

Best, Charles Herbert, 1950 (I), Banting and Best Department of Medical Research, University of Toronto, Toronto, Canada

Bjerknes, V. (F. K.), 1934 (F), The University, Oslo, Norway

Bohr, Niels, 1925 (C), Institute for Theoretical Physics, Blegdamsvej 15, Copenhagen, Denmark

Bordet, Jules, 1935 (I), Pasteur Institute, Rue du Remorqueur, 28, Brussels, Belgium

Bragg, Sir William Lawrence, 1945 (C), Cavendish Laboratory, The University, Cambridge, England

de Broglie, Prince Louis, 1948 (C), 94 Rue Perronet, Neuilly-sur-Seine, France

Cartan, Élie, 1949 (A), University of Paris, Paris, France

Caso, Alfonso, 1943 (K), Secretaria de Bienes Nacionales e Inspeccion Administrativa, Mexico, D. F.

Chapman, Sydney, 1946 (A), Letters: Queen's College, Oxford; Printed matter: The Mathematical Institute, Oxford, England

Dale, Sir Henry Hallett, 1940 (I), The Wellcome Trust, 28 Portman Square, London, W.1, England

Debye, Peter, 1931* (C), Baker Laboratory, Cornell University, Ithaca, N. Y. (U.S.A.)

Dirac, Paul Adrien Maurice, 1949 (A), Department of Mathematics, St. John's College, Cambridge, England

Einstein, Albert, 1922† (C), The Institute for Advanced Study, Princeton, N. J. (U.S.A.)

Fisher, Ronald Aylmer, 1948 (H), Department of Genetics, University of Cambridge, 44 Storey's Way, Cambridge, England

Hadamard, Jacques, 1926 (A), 12, rue Emile Faguet, Paris XIV, France

---

* Dr Debye became a naturalized citizen in 1946 and a member of the Academy in 1947.

† Dr. Einstein became a naturalized citizen in 1941 and a member of the Academy in 1942.

Helland-Hansen, Björn, 1947 (F), Chr. Michelsens Institutt for Videnscap, Bergen, Norway

Hill, Archibald Vivian, 1941 (I), 16 Bishopswood Road, Highgate, London, N.6, England

Hill, James Peter, 1940 (H), Kanimbla, Dollis Avenue, London N.3, England

Houssay, Bernardo Alberto, 1940 (I), Viamonte 2790, Buenos Aires, Argentina

Jeffreys, Harold, 1945 (B), St. John's College, Cambridge, England

Jones, Sir Harold Spencer, 1943 (B), Royal Greenwich Observatory, Herstmonceux Castle, Hailsham, Sussex, England

Kapitza, Peter Leonidovich, 1946 (C), Institute for Physical Problems, Academy of Sciences of the U.S.S.R., Moscow, U.S.S.R.

Karrer, Paul, 1945 (E), University of Zurich, Zurich, Switzerland

Keith, Sir Arthur, 1941 (I, K), Buckston Browne Farm, Downe, Farnborough, Kent, England

Kluyver, Albert Jan, 1950 (G), Technical University, Delft, The Netherlands

Levi, Giuseppe, 1940 (H), Instituto di Anatomia Umana, Corso Massimo D'Azeglio, 52, Turin, Italy

Lim, Robert K. S., 1942 (I), Army Medical Administration, Municipal Government, Shanghai, China

Linderstrøm-Lang, Kaj Ulrik, 1947 (E), Chemical Division, Carlsberg Laboratory, Copenhagen, Denmark

Lyot, Bernard Ferdinand, 1949 (B), 9 bis rue Boileau, Paris 16, France

Pieron, Henri, 1949 (K), Institute of Psychology, University of Paris, Paris, France

Robinson, Sir Robert, 1934 (E), Dyson Perrins Laboratory, South Parks Road, Oxford, England

Ruzicka, Leopold, 1944 (E), Department of Organic Chemistry, Institute of Technology, Zurich, Switzerland

Sherrington, Sir Charles Scott, 1924 (I), Gonville and Caius College, Cambridge, England

Sommerfeld, Arnold, 1929 (C), Dunant-strasse 6, Munich 23, Germany

Southwell, Richard Vynne, 1943 (D), Imperial College of Science and Technology, South Kensington, London, S.W.7, England

Svedberg, The, 1945 (E), Fysikalisk-Kemiska Institutionen, University of Uppsala, Uppsala, Sweden

Taylor, Sir Geoffrey I., 1945 (A), Trinity College, Cambridge, England

Tiselius, Arne W. K., 1949 (I), Institute of Biochemistry, Uppsala University, Uppsala, Sweden

Vallee-Poussin, C. de la, 1929 (A), University of Louvain, Louvain, Belgium

Vening Meinesz, Felix Andries, 1939 (F), Potgieterlaan 5, Amersfoort, The Netherlands

Watson, D. M. S., 1938 (H), University College, Gower Street, London, W.C.1, England

Wieland, Heinrich, 1932 (E), Sophienstrasse 9, Munich 2 NW, Germany
Winge, Öjvind, 1949 (I), Department of Physiology, Carlsberg Laboratory, Copenhagen (Valby), Denmark
Yukawa, Hideki, 1949 (C), Department of Physics, Columbia University, New York 27, N. Y. (U.S.A.)

Number of Foreign Associates July 1, 1950: 46.

If a foreign associate becomes a member of the Academy his name is not counted in the limit of 50 foreign associates.

SECTIONS

(1) *Mathematics*—28 members

Zariski, Oscar, *Chairman* (1952)
Albert, A. A.
Alexander, J. W.
Bell, E. T.
Bliss, G. A.
Bochner, S.
Coble, A. B.
Douglas, Jesse
Eisenhart, L. P.

Evans, G. C.
Kasner, Edward
Lefschetz, Solomon
McShane, E. J.
Mac Lane, Saunders
Miller, G. A.
Moore, R. L.
Morse, Marston
Murnaghan, F. D.
Ritt, J. F.

Smith, Paul A.
Stone, M. H.
Thomas, T. Y.
Vandiver, H. S.
Veblen, Oswald
von Neumann, John
Walsh, J. L.
Weyl, Hermann
Whitney, Hassler

(2) *Astronomy*—28 members

Merrill, P. W., *Chairman* (1953)
Abbot, C. G.
Adams, W. S.
Aitken, R. G.
Babcock, H. D.
Bowen, I. S.
Fleming, J. A.
Hubble, E. P.
Joy, A. H.

King, A. S.
Kuiper, G. P.
Lambert, W. D.
Leuschner, A. O.
Mayall, N. U.
Menzel, D. H.
Mitchell, S. A.
Moulton, F. R.
Nicholson, S. B.
Ross, F. E.

Russell, H. N.
Seares, F. H.
Shapley, Harlow
Slipher, V. M.
Stebbins, Joel
Struve, Otto
Trumpler, R. J.
Wilson, Ralph E.
Wright, W. H.

(3) *Physics*—65 members

Beams, J. W., *Chairman* (1951)
Allison, S. K.
Alvarez, L. W.
Anderson, C. D.
Bacher, R. F.
Bainbridge, K. T.
Berkner, L. V.
Bethe, H. A.
Birge, R. T.
Bjerknes, Jacob

Bloch, Felix
Breit, Gregory
Bridgman, P. W.
Brode, R. B.
Coblentz, W. W.
Compton, A. H.
Compton, K. T.
Condon, E. U.
Coolidge, W. D.
Crew, Henry
Davis, Bergen

Davisson, C. J.
DuBridge, L. A.
Dunning, J. R.
Einstein, Albert
Epstein, P. S.
Fermi, Enrico
Franck, James
Goudsmit, S. A.
Houston, W. V.
Hull, A. W.
Ives, H. E.

Kemble, E. C.
Lauritsen, C. C.
Lawrence, E. O.
Loomis, F. W.
Lyman, Theodore
McMillan, E. M.
Mason, Max
Millikan, R. A.
Mulliken, R. S.
Nier, A. O. C.
Oppenheimer, J. R.

Pegram, G. B.
Pierce, G. W.
Piggot, C. S.
Rabi, I. I.
Reichelderfer, F. W.
Rossby, C.-G.
Rossi, Bruno
Saunders, F. A.
Schwinger, Julian
Slater, J. C.
Slichter, L. B.

Stern, Otto
Stewart, G. W.
Sverdrup, H. U.
Teller, Edward
Tuve, M. A.
Van Vleck, J. H.
Webster, D. L.
Wigner, E. P.
Wilson, Edwin B.
Wood, R. W.
Zachariasen, W. H.

(4)   *Engineering*—36 members

Hunsaker, J. C.,
 Chairman (1953)
Adams, C. A.
Briggs, L. J.
Buckley, O. E.
Bush, Vannevar
Cochrane, E. L.
Curme, G. O., Jr.
Dryden, H. L.
Dunn, Gano
Durand, W. F.
Fletcher, Harvey
Foote, P. D.

Gibbs, W. F.
Gilliland, E. R.
Herty, C. H., Jr.
Hoover, Herbert
Jeffries, Zay
Kelly, M. J.
Kettering, C. F.
Lewis, W. K.
Loomis, A. L.
Merica, P. D.
Murphree, E. V.
Raymond, A. E.

Savage, J. L.
Slepian, Joseph
Soderberg, C. R.
Stratton, J. A.
Suits, C. G.
Terman, F. E.
Thomas, C. A.
Timoshenko, Stephen
von Karman, T.
Whitehead, J. B.
Wilson, Robert E.
Zworykin, V. K.

(5)   *Chemistry*—62 members

Fuson, R. C., *Chairman* (1953)
Adams, L. H.
Adams, Roger
Bachmann, W. E.
Bancroft, W. D.
Bartlett, P. D.
Baxter, G. P.
Bogert, M. T.
Bolton, E. K.
Conant, J. B.
Cope, A. C.
Craig, L. C.
Daniels, Farrington
Debye, Peter
Elderfield, R. C.
Eyring, Henry
Fieser, L. F.
Folkers, Karl
Giauque, W. F.
Gilman, Henry

Hammett, L. P.
Harkins, W. D.
Harned, H. S.
Hildebrand, J. H.
Hudson, C. S.
Ipatieff, V. N.
Jacobs, W. A.
Johnson, J. R.
Keyes, F. G.
Kharasch, M. S.
Kirkwood, J. G.
Kistiakowsky, G. B.
Kraus, C. A.
La Mer, V. K.
Lamb, A. B.
Langmuir, Irving
Latimer, W. M.
Libby, W. F.
Lind, S. C.
Longsworth, L. G.
McElvain, S. M.

MacInnes, D. A.
Marvel, C. S.
Mayer, J. E.
Mees, C. E. K.
Noyes, W. A., Jr.
Onsager, Lars
Pauling, Linus
Pitzer, K. S.
Rodebush, W. H.
Scatchard, George
Schlesinger, H. I.
Seaborg, G. T.
Small, L. F.
Smith, L. I.
Urey, H. C.
Williams, Robert R.
Williams, Roger J.
Wilson, E. Bright, Jr.
Wulf, O. R.
Wyckoff, R. W.
Yost, D. M.

(6) *Geology*—39 members

Rubey, W. W., *Chairman* (1951)
Allen, E. T.
Berkey, C. P.
Birch, Francis
Blackwelder, Eliot
Bowen, N. L.
Bradley, W. H.
Bucher, W. H.
Buddington, A. F.
Byerly, Perry
Chaney, R. W.
Cloos, Ernst
Daly, R. A.

Day, A. L.
Dunbar, C. O.
Ewing, Maurice
Gilluly, James
Gregory, W. K.
Gutenberg, Beno
Hewett, D. F.
Kelley, W. P.
Knopf, Adolph
Larsen, E. S., Jr.
Lawson, A. C.
Leith, C. K.
Longwell, C. R.

Lovering, T. S.
Macelwane, J. B.
Mead, W. J.
Mendenhall, W. C.
Palache, Charles
Reeside, J. B., Jr.
Ruedemann, Rudolf
Simpson, G. G.
Stock, Chester
Vaughan, T. W.
Williams, Howel
Woodring, W. P.
Wright, F. E.

(7) *Botany*—37 members

Jones, D. F., *Chairman* (1953)
Babcock, E. B.
Bailey, I. W.
Bailey, L. H.
Beadle, G. W.
Blakeslee, A. F.
Bonner, James
Brink, R. A.
Burkholder, P. R.
Campbell, D. H.
Chandler, W. H.
Cleland, R. E.

Couch, J. N.
Delbrück, Max
Dodge, B. O.
Duggar, B. M.
Fred, E. B.
Goddard, D. R.
Kunkel, L. O.
McClintock, Barbara
Mangelsdorf, P. C.
Merrill, E. D.
Osterhout, W. J. V.
Raper, K. B.
Rhoades, M. M.

Robbins, W. J.
Sax, Karl
Sinnott, E. W.
Smith, Gilbert M.
Stadler, L. J.
Stakman, E. C.
Thimann, K. V.
Thom, Charles
Van Niel, C. B.
Waksman, S. A.
Walker, J. C.
Went, F. W.

(8) *Zoology and Anatomy*—41 members

Romer, Alfred S., *Chairman* (1952)
Bartelmez, G. W.
Bigelow, H. B.
Castle, W. E.
Conklin, E. G.
Corner, G. W.
Danforth, C. H.
Demerec, Milislav
Detwiler, S. R.
Dobzhansky, Theodosius
Dunn, L. C.
Goldschmidt, R. B.
Harrison, R. G.

Hartman, C. G.
Harvey, E. N.
Herrick, C. J.
Hisaw, F. L.
Hutchinson, G. E.
Irwin, M. R.
Jacobs, M. H.
Lewis, W. H.
Metz, C. W.
Moore, C. R.
Muller, H. J.
Nicholas, J. S.
Painter, T. S.
Parker, G. H.

Patterson, J. T.
Riddle, Oscar
Schmitt, F. O.
Smith, Philip E.
Sonneborn, T. M.
Stern, Curt
Sturtevant, A. H.
Taliaferro, W. H.
Twitty, V. C.
Weiss, Paul
Wetmore, Alexander
Willier, B. H.
Wislocki, G. B.
Wright, Sewall

(9) *Physiology and Biochemistry—55 members*

Doisy, E. A., *Chairman* (1951)
Anderson, R. J.
Ball, E. G.
Bard, Philip
Bronk, D. W.
Carlson, A. J.
Clark, W. M.
Clarke, H. T.
Cohn, E. J.
Cori, Carl F.
Cori, Gerty T.
Davis, Hallowell
DuBois, E. F.
duVigneaud, Vincent
Elvehjem, C. A.
Erlanger, Joseph
Evans, H. M.
Fenn, W. O.

Forbes, Alexander
Gasser, H. S.
Hart, E. B.
Hartline, H. K.
Hastings, A. B.
Kendall, E. C.
Lewis, H. B.
Link, K. P.
Lipmann, Fritz
Loeb, R. F.
Long, C. N. H.
Lorente de Nó, R.
McCollum, E. V.
MacNider, W. deB.
Mann, F. C.
Marshall, E. K., Jr.
Maynard, L. A.
Meek, W. J.
Meyerhof, Otto

Northrop, J. H.
Oncley, J. L.
Peters, J. P.
Richards, A. N.
Rose, W. C.
Schmidt, C. F.
Shaffer, P. A.
Sherman, H. C.
Smith, Homer W.
Stadie, W. C.
Stanley, W. M.
Sumner, J. B.
Van Slyke, D. D.
Vickery, H. B.
Wald, George
Werkman, C. H.
Wintersteiner, Oskar
Wolfrom, M. L.

(10) *Pathology and Bacteriology—38 members*

Dochez, A. R., *Chairman* (1951)
Armstrong, Charles
Avery, O. T.
Blake, F. G.
Blalock, Alfred
Cannon, P. R.
Castle, W. B.
Coggeshall, L. T.
Cole, Rufus
Dragstedt, L. R.
Dubos, R. J.
Francis, Thomas, Jr.

Gamble, J. L.
Goodpasture, E. W.
Graham, E. A.
Heidelberger, Michael
Horsfall, F. L., Jr.
Huggins, C. B.
Keiser, R. A.
Little, C. C.
Loeb, Leo
Long, E. R.
Longcope, W. T.
Maxcy, K. F.
Meyer, K. F.

Mueller, J. Howard
Murphy, J. B.
Novy, F. G.
Opie, E. L.
Paul, J. R.
Rivers, T. M.
Robertson, O. H.
Rous, Peyton
Sabin, Florence R.
Shope, R. E.
Tyzzer, E. E.
Whipple, G. H.
Wolbach, S. B.

(11) *Anthropology—10 members*

Linton, Ralph, *Chairman* (1951)
Hooton, E. A.
Kidder, A. V.

Kroeber, A. L.
Lowie, R. H.
Schultz, A. H.
Shapiro, H. L.

Spier, Leslie
Swanton, J. R.
Tozzer, A. M.

(12) *Psychology—22 members*

Carmichael, Leonard, *Chairman* (1953)
Beach, F. A.
Boring, E. G.
Gesell, Arnold

Graham, C. H.
Hilgard, E. R.
Hull, C. L.
Hunter, W. S.
Köhler, Wolfgang

Lashley, K. S.
Miles, W. R.
Pillsbury, W. B.
Richter, C. P.
Skinner, B. F.

Stevens, S. S.	Thurstone, L. L.	Woodworth, R. S.
Stone, C. P.	Tolman, E. C.	Yerkes, R. M.
Terman, L. M.	Wever, E. G.	

Temporary Nominating Group on Geophysics—1942–51

30 members

John A. Fleming, *Chairman*

Mathematics: F. D. Murnaghan.

Astronomy: C. G. Abbot, J. A. Fleming, Walter D. Lambert, Harlow Shapley.

Physics: L. V. Berkner, Jacob Bjerknes, P. W. Bridgman, A. H. Compton, C. S. Piggot, F. W. Reichelderfer, C.-G. Rossby, L. B. Slichter, H. U. Sverdrup.

Engineering: L. J. Briggs, J. C. Hunsaker.

Chemistry: L. H. Adams, S. C. Lind, Oliver R. Wulf.

Geology: Francis Birch, Perry Byerly, Arthur L. Day, Maurice Ewing, Beno Gutenberg, Adolph Knopf, J. B. Macelwane, T. Wayland Vaughan, F. E. Wright.

Botany: S. A. Waksman.

Zoology and Anatomy: H. B. Bigelow.

COMMITTEES

Auditing

Merle A. Tuve, *Chairman;* E. U. Condon, Hugh L. Dryden.

Biographical Memoirs

Detlev W. Bronk, *Chairman, ex officio,* President of the Academy.
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(Joint Committee of the Academy and Research Council)
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Discoveries in physical, or astronomical science or novel application of science to purposes beneficial to the human race. (Every five years the committee recommends the person whom they consider the most deserving of the medal, and upon approval by the Academy, the name of the nominee is forwarded to the trustees of Columbia University, who administer the Barnard Medal Fund.)

Committee: Ernest O. Lawrence, *Chairman;* Edwin Hubble, I. I. Rabi, V. M. Slipher, W. R. Whitney.

John J. Carty Fund

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Committee: Herbert Gasser, *Chairman* (1952); Roger Adams (1954), Harvey Fletcher (1951), E. C. Stakman (1955), Robert E. Wilson (1953)

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Committee: I. I. Rabi, *Chairman* (1952); J. W. Beams (1951), P. W. Bridgman (1955), Arthur H. Compton (1954), Enrico Fermi (1953).

Henry Draper Fund

Medal and investigations in astronomical physics. $10,000.

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Daniel Giraud Elliot Fund

Medal and honorarium for most meritorious work in zoology or paleontology published each year. $8,000.

Committee: Alexander Wetmore, *Chairman* (as Secretary, Smithsonian Institution); Albert E. Parr (as Scientific Director, American Museum of Natural History); Carl O. Dunbar (1953), A. H. Sturtevant (1951).

Wolcott Gibbs Fund

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Joseph Henry Fund

To assist meritorious investigators, especially in the direction of original research. $47,163.50.
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Kovalenko Fund

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Marsh Fund

Original research in the natural sciences. $20,000.
Committee: C. S. Marvel, *Chairman* (1954); A. F. Buddington (1951), W. M. Stanley (1953), W. H. Taliaferro (1952), F. E. Terman (1955).

Murray Fund

Agassiz Medal for original contribution in the science of oceanography. $10,000.
Committee: Henry B. Bigelow, *Chairman* (1953); Maurice Ewing (1952), W. W. Rubey (1951).

J. Lawrence Smith Fund

Medal and investigations of meteoric bodies. $10,000.
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(1955), Harlow Shapley (1953), Joel Stebbins (1954), R. J. Trumpler (1951).

Mary Clark Thompson Fund

Medal for most important services to geology and paleontology. $10,000.
Committee: Ralph W. Chaney, *Chairman* (1952); W. H. Bradley (1953); G. G. Simpson (1951).

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Medal and honorarium to stimulate research in pre-Cambrian or Cambrian life. $5,000.
Board of Trustees: Carl O. Dunbar, *Chairman;* Alexander Wetmore, *ex officio,* Secretary, Smithsonian Institution; Pierre Pruvost, representing the Institut de France (1952); C. J. Stubblefield, representing The Royal Society of London (1952); John B. Reeside, Jr. (1953).

James Craig Watson Fund

Medal and the promotion of astronomical research. $25,000.
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THE EFFECT OF IRREGULAR ABSORPTION ON GALAXY DISTRIBUTION

By Constance Warwick

Harvard College Observatory, Cambridge, Massachusetts

Introduction and Method of Analysis.—Recent researches[1-4] on interstellar absorbing material have emphasized the irregularity of its distribution. Greenstein[5] has found that the obvious large clouds near the Milky Way have diameters of the order of 100 pc., and photographic absorptions of about 1 mag., but that they account for only about one-ninth of the observed absorption. Spitzer,[6] summarizing recent investigations of smaller dark clouds assumed to be responsible for the remaining absorption, quotes the following values: average diameter, 16 pc.; mean photographic absorption per cloud, $0.^m23$; space density, between 1.0×10^{-5} and 1.2×10^{-4} clds./pc.^{3}.

The apparent distribution of galaxies offers an opportunity to study these small dark clouds. The methods outlined by Sterne[7] have been used to analyze the galaxy counts of Hubble.[8] The observations consist of galaxy counts made on plates taken at the Mount Wilson Observatory, and reduced to uniform ideal observing conditions. The fields are spaced at intervals of 5° in galactic latitude and approximately 10° in longitude, and each has an area of 0.6 square degree.

If there are discrete absorbing clouds, each with angular diameter large enough to cover two or more of the fields in which galaxy counts have been made, there will be a relation between the numbers of galaxies in adjacent fields. At each galactic latitude, we find for each field the residual from the mean number of galaxies per field for that latitude. The association test indicates whether the pattern of positive and negative residuals differs significantly from the pattern which would result if there were no irregular absorption. The correlation test is similar, but considers the size of the residuals, as well as the pattern.

The results of the tests are expressed by the value of P, the probability

that a value of the association (correlation) coefficient, equally or less probable than the value observed, should result from a random distribution. Thus $P = 1.00$ means that the observed distribution cannot be distinguished from a random distribution, while a very small value of P indicates that the observed distribution could hardly occur in the absence of some external effect on the numbers being analyzed. A value of P greater than 0.10, indicating that there is one chance in ten, or better, of finding the observed association in a random distribution, is not considered significant. The line between random and significantly non-random distributions is conventionally drawn at $P = 0.05$, while a value of P less than 0.02 may be considered to suggest strongly a departure from the laws of chance.

In applying the tests to the Mount Wilson galaxy counts, the fields at each galactic latitude were treated separately. The mean number of galaxies per field, $\bar{N}$, was found for the latitude zone, and the residual $x = (N - \bar{N})$ found for each field. The mean value, y, of the residual for the two adjacent fields was found, and the association and correlation coefficients between the values of x and y computed.

Let $N(x+)$ indicate the number of fields with positive residual x, $N(x+, y+)$ the number with positive x *and* positive y, etc. The association is clearly seen by making a table. The appropriate association coefficient

y \ x	$+$	$-$	
$+$	$N(x-, y+)$	$N(x-, y+)$	$N(y+)$
$-$	$N(x+, y-)$	$N(x-, y-)$	$N(y-)$
	$N(x+)$	$N(x-)$	n

has been given by Sterne.[7] In the present notation it is:

$$R = \frac{N(x+, y+) \cdot N(x-, y-) - N(x+, y-) \cdot N(x-, y+)}{\sqrt{\dfrac{N(x+) \cdot N(x-) \cdot N(y+) \cdot N(y-)}{(n - 1)}}},$$

where n is the total number of fields in the latitude zone. Then $P(R)$ is given approximately by the area under the normal error curve corresponding to values of the abcissa numerically equal or greater than R. The correlation coefficient is given by

$$r = \frac{\sum xy}{\sqrt{\sum x^2 \cdot \sum y^2}}.$$

The value of $P(r)$ can be found from tables[8] if n is less than 30. If n is

equal or greater than 30, we take $P(r)$ equal to the area under the normal error curve corresponding to values of the abscissa numerically equal or greater than t where

$$t = \frac{r}{\sqrt{1 - r^2}} \cdot \sqrt{n - 2}.$$

The significance of the tests depends on the linear diameter of the smallest cloud that can be detected with this observational material. A rough estimate, assuming the space density of the clouds to be known, shows that the association test is not sensitive to the presence of clouds with linear diameter smaller than about 15 pc.

The Distribution at High Latitudes.—In order to separate intrinsic irregularities in the distribution of galaxies from irregularities caused by absorption, the data for galactic latitudes $\pm40°$ to $\pm60°$ were first examined. The frequency distribution of the numbers of galaxies per field was compared to the Poisson distribution, and found to have a much greater dispersion and definite asymmetry, with an excessive number of large positive residuals. (See Fig. 3, p. 7 of reference 10.) Association and correlation tests were applied to the high latitude distribution, and gave $P = 0.99$ and 0.62, respectively. From these results we conclude, first, that the large dispersion in the numbers of galaxies per field at high latitudes is caused largely by clustering, although perhaps partly by accidental magnitude errors and by the systematic correction of the actual counts; second, that the "clusters" are not large enough to affect more than a single field; and finally, that there are no dark nebulae present at these latitudes with large enough angular diameter to affect more than one field.

The Latitude Zones $+15°$ to $+30°$ and $-15°$ to $-30°$.—The number of fields with each value of log N was plotted against log N for each latitude zone (Fig. 1). In order to include in the frequency diagrams the fields in which no galaxies were found, the maximum possible value of log N was computed for each of the fields, assuming a uniform distribution of galaxies, and the density given by Hubble. For comparison with the observed curves, the theoretical distribution was computed assuming: (1) that the absorption is uniform (full drawn curves); and (2) that the absorption is caused by discrete clouds, each absorbing $0\overset{m}{.}23$, which are distributed over the sky according to Poisson's law (broken curves).

In the case of uniform absorption, the dispersion of the frequency distribution of the logarithms will increase as the number of galaxies per field decreases. The magnitude of this effect can be roughly estimated. If N_1 is the counted number in a given field, N the corrected number and a the correction factor, $N = aN_1$. For a random distribution, the dispersion in the counted number is equal to $\sqrt{N_1}$, and the dispersion in the cor-

rected number is $\bar{a}\sqrt{\bar{N_1}} \sim \sqrt{\bar{a}\bar{N}}$. The dispersion in the values of log N is approximately $0.43/\bar{N}$ times the dispersion in the values of N, or 0.43 $\sqrt{\bar{a}/\bar{N}}$. Since the frequency distribution of the logarithms of the numbers of galaxies per field for high latitudes is so closely represented by a normal distribution, we assume that the actual distribution of the logarithms is the result of two normal distributions, one, with dispersion s_0, caused by clustering and accidental errors, superimposed on the other with dispersion s_1 equal to the "natural uncertainty." s_0 is independent of galactic latitude, while s_1 increases with decreasing latitude. For the distribution resulting from the combination, the dispersion, s, will be given by

$$s^2 = s_0^2 + s_1^2 = s_0^2 + \frac{0.188\bar{a}}{\bar{N}}. \tag{1}$$

The value of s_0^2 was found by substituting in (1) the values of s and $\bar{N}$ observed for the high latitude region. The value of s for each latitude was then found from (1). The normal error curve with dispersion s and mean log N for the latitude zone is the predicted frequency curve for uniform absorption.
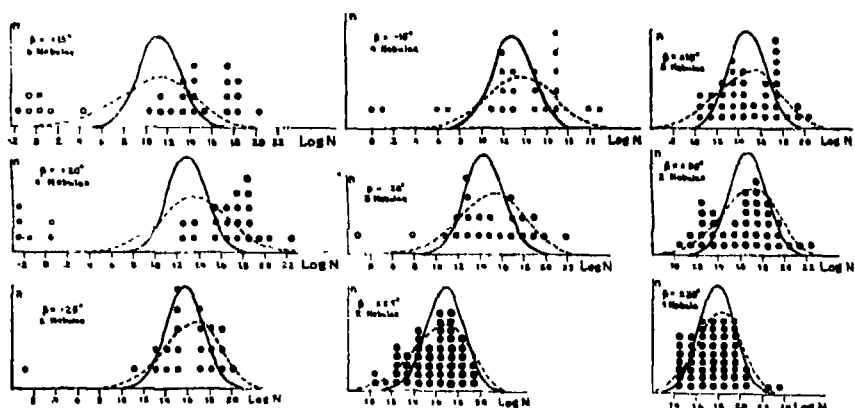
FIGURE 1

A Poisson distribution of the number of dark clouds along the line of sight was assumed in predicting the frequency of values of log N for the case that small dark clouds cause all the observed absorption. The mean value, $\Delta m(\beta)$, of absorption for each latitude zone was found from the relation

$$\log N(\beta) = \log N_0 - 0.6 \Delta m(\beta), \tag{2}$$

where log N_0 is the mean value of log N in the absence of absorption (log N for the high latitude region), and log $N(\beta)$ is the mean value for the latitude zone under consideration. The mean number of dark clouds along the line of sight was taken as the nearest integer to $\Delta m/0\overset{m}{.}23$. The number of fields affected by 0, 1, 2, ... clouds was computed according to Poisson's law for each value of mean number of clouds along the line of sight. The frequency curve of log N for $\pm 40°$ to $\pm 60°$ was "smeared out" according to the Poisson distribution to give the predicted frequency curve.

A comparison of the predicted and observed frequency curves shows that neither hypothesis, small clouds or uniform absorption, explains the observed frequency when the fields in the zone of avoidance are included.

TABLE 1

All Fields

Association	$P > 0.10$	$0.05-0.10$	$0.02-0.05$	$0.01-0.02$	<0.01
	$-15°$		$+15°$		$\pm 20°$
	$-25°$		$+25°$		$\pm 30°$
Correlation	$-15°$	$+30°$			$+15°$
	$-25°$				$\pm 20°$
					$+25°$
					$-30°$

Omitting Fields in Zone of Avoidance					
Association	$\pm 15°$		$+25°$		$\pm 20°$
	$-25°$				$\pm 30°$
Correlation	$-15°$	$+30°$	$+15°$		$+25°$
	$+20°$		$-20°$		$-30°$
	$-25°$				

As would be expected from Greenstein's work, we must assume larger clouds, or non-random accumulations of small clouds, to explain the zone of avoidance. When the fields in the zone of avoidance are omitted, the distribution of values of log N at positive and negative latitudes is sufficiently similar that we may combine $+15°$ with $-15°$, etc. The curves are shown in figure 1. We have not given the hypothesis of discrete clouds a completely fair chance to represent the observations, because the mean number of clouds was taken to be integral, so that the mean absorption for the predicted curve may not agree exactly with the observed mean. From these curves it appears that the discrete cloud hypothesis gives slightly better agreement with observation. More galaxy counts at low latitudes are needed to settle the question definitely.

The association and correlation tests were carried out as before, first including the fields in the zone of avoidance, and then omitting them. The results are given in table 1. We find that, even when the fields in the zone of avoidance are omitted, there is considerable evidence that the absorbing matter is not completely uniform. This may indicate the existence of clouds, or it may be caused by the fact that the absorption is stronger in the direction of the galactic center than in the opposite part of the sky. The data were corrected for this longitude effect, and the tests repeated. The correction was determined for each latitude (grouping $+15°$ with $-15°$, etc.) by plotting the residual $(N - \bar{N})$ against cos $\frac{1}{2}(\lambda - \lambda_0)$, where λ is the galactic longitude of the field, and λ_0, the longitude of the galactic center, was taken as $325°$. A straight line was drawn through the points, from which the mean residual at each longitude was found and subtracted from the residual for the field. The corrected residuals (with fields in the zone of avoidance omitted) show association with $P > 0.10$ for all latitude zones except that at $+25°$, which has $0.02 < P \leq 0.05$. The correlation test gives $P < 0.01$ for the zone at $30°$, $0.02 < P \leq 0.05$ for those at $+15°$ and $+25°$, and $P > 0.10$ for all other zones. From these results we conclude that the small dark clouds, if they exist, are smaller than the critical diameter, 15 pc., to which the tests are sensitive.

The Dispersion at Low Latitudes.—The frequency distribution of log N shows that the observed dispersion is generally greater than that predicted for uniform absorption. The observed dispersions in the values of log N were tested to find the probability that as large or larger dispersion be found if the absorption is uniform. The dispersion for uniform absorption, s, was predicted by equation (1). If s' is the observed dispersion, and n the number of fields, $\chi^2 = s'^2/s^2$, and the desired probability, $P(\chi^2)$, can be found from tables[9] when $n \leq 30$. If $n > 30$, we compute $m = \sqrt{2\chi^2} - \sqrt{(2n - 3)}$, and use the approximation

$$P = \frac{2}{\sqrt{2\pi}} \int_{|m|}^{\infty} e^{-x^2/2}\, dx.$$

The dispersion depends on the number of dark nebulae along the line of sight, and so, since the total absorption is known, on the value of τ, the absorption of a single cloud. We estimate the minimum value of τ which will result in a significant dispersion. Suppose that, in a given latitude zone, values of the number of dark clouds along the line of sight, n, are distributed about the mean, $\bar{n}$, according to Poisson's law, with dispersion $\sqrt{\bar{n}}$. Substituting in (2), $\Delta m(\beta) = n(\beta)\tau$, we find

$$\log N(\beta) = \log N_0 - 0.6 n(\beta)\tau.$$

The dark clouds cause a dispersion in log N equal to $0.6\,\tau\sqrt{\bar{n}(\beta)}$. We find for the observed dispersion, using (1);

$$s^2 = s_0^2 + \frac{0.188\bar{d}}{\bar{N}} + 0.36 \, \overline{\Delta m(\beta)}\tau. \tag{3}$$

Substituting values of the constants for $+15°$, we find that if $P \leq 0.10$, $\tau \geq 0.''05$.

The dispersion test was made only on the residuals in log N corrected for the longitude effect. Fields for which the correction factor exceeded 3.5, and those noted by Hubble as in the zone of avoidance or containing a cluster were omitted. For the zone at $-15°$, $P < 0.01$, while $0.02 < P \leq 0.05$ at $+15°$, and $0.05 < P \leq 0.10$ at $-20°$; $P > 0.10$ for all other zones.

Recomputation of Value of τ.—In his analysis of the Mount Wilson galaxy counts, Ambarzumian[2] has made the tacit assumption that the dispersion in the number of galaxies per field is caused entirely by irregularities in absorption. A repetition of his analysis, taking into account the tendency of galaxies to cluster, gives a value of τ, the mean absorption of one dark cloud, considerably smaller than that found by Ambarzumian.

The values of log N given in Mount Wilson Contribution 485 for latitudes $\pm 15°$ to $\pm 85°$ were used in the analysis, omitting fields for which the correction factor exceeds 3.5, fields in or near the zone of avoidance, and fields containing conspicuous clusters. (See table XI, p. 41 of reference 8.) The mean value of s^2, the square of the observed dispersion in log N for a given zone, was plotted against csc $|\beta|$ for each $|\beta|$. Assuming the relation $s^2 = a + b$ csc $|\beta|$, a least squares solution was made for the constants a and b. The solution depends strongly on the values for $\pm 15°$ and $\pm 20°$; the values for higher latitudes show no relation to the latitude.

In (3) we substitute $\overline{\Delta m(\beta)} = 0.25$ csc $|\beta|$ and log $N(\beta) = \log N_0 - 0.6 \Delta m(\beta)$. Using the approximation $N^{-1} = N_0^{-1} 10^{0.15 \, \text{csc} \, |\beta|}$ and using the first two terms of the Taylor expansion, we get

$$s^2 = s_0^2 + \frac{0.188}{N_0} + \left[\frac{0.188}{N_0} \cdot \frac{0.15}{0.43} + 0.09\tau\right] \text{csc} \, |\beta|.$$

Setting the coefficient of csc $|\beta|$ equal to 0.00823, the value of b found from the least squares solution, and substituting $N_0 = 82.4$, we find $\tau = 0.''08$.

The smaller value of τ leads to different values of the other constants which describe the distribution of the absorbing matter, assuming that it occurs in the form of small clouds. Spitzer gives the relations

$$A = k\tau \tag{4}$$

$$k = 1000\pi r^2 D, \tag{5}$$

where A is the total photographic absorption in magnitudes per kiloparsec, k is the number of clouds per kiloparsec along a line of sight, D is the number of clouds per cubic parsec and r is the average radius of a cloud. If we assume that $0.''1$ is an upper limit for the value of r, then we find from (4) that 16 is a lower limit for k. If r is as small as $0.''1$, the effect of a single cloud on a galaxy field will not be distinguishable from random variations in the numbers of galaxies, and our conclusions from the association and correlation tests as to the size of the clouds are meaningless. However, let us assume that the average radius is 10 pc. Then we find that D, the number of clouds per cubic parsec, is at least as great as 5×10^{-6}, and that the average distance between clouds is only about 27 pc. Acceptance of these conclusions from the galaxy distribution leads us to a picture very close to uniformity.

Galaxy counts over large areas in low galactic latitudes would greatly increase the value of this sort of analysis. Such data should smooth out the observed frequency distribution curves, so that a trial and error fitting of a Poisson distribution would indicate the mean value of absorption of a ·single cloud. These counts would add to the sensitivity, as well as to the significance of the association and correlation tests. By grouping areas of one square degree into larger areas, and finding at which area the association enters, an estimate of the mean diameters would be possible.

Conclusions.—(1) The distribution of galaxies between galactic latitudes $\pm 40°$ and $\pm 60°$ shows that there are clusters of galaxies, but there is no evidence of irregular absorption at these latitudes.

(2) The frequency distributions of log N suggest that the absorbing material at $\pm 15°$ to $\pm 30°$ and outside the zone of avoidance may be condensed into small clouds, but more observations are needed to show definitely that the absorption is not uniform. The large dark nebulae of the Milky Way cannot be explained as random accumulations of small clouds.

(3) The positive association and correlation between residuals in neighboring fields are caused at least partly by the fact that the absorption is stronger toward the galactic center than toward the anti-center. If the absorbing material occurs mainly in clouds, they are not larger than about 15 pc. in diameter.

(4) The dispersion in values of log N does not differ significantly from that predicted for uniform absorption, indicating that the value of r, the absorption of a single cloud, is not much greater than $0.''05$. A recomputation of the value of r, following the method of Ambarzumian, but considering the clustering of galaxies and the increase in dispersion due to uniform absorption, gives $r = 0.''08$.

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THE MECHANISM OF ACTION OF A BACTERIAL TOXIN ON PLANT CELLS

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An attempt has been made in this investigation to account in biochemical terms for a toxemia associated with the wildfire disease of tobacco. Wildfire, a bacterial disease, the causal agent of which is *Pseudomonas tabaci*, is characterized by localized chlorotic halos 1 to 2 cm. in diameter (Fig. 1a) that surround a central brown necrotic spot that is usually but not always quite small in size. The chlorotic area is free of bacteria and results from the diffusion of a toxic substance secreted by the bacteria present in the central necrotic focus of infection. In young tobacco plants the toxin may become systemic, diffusing throughout the plants and frequently killing them. Losses resulting from seedbed infection of the disease may thus be very high.

P. tabaci produces the toxin freely on a variety of culture media as well as in the host.[1-3] This substance can be separated from the bacteria by filtration and the sterile culture filtrates are capable of reproducing the toxic manifestations of the wildfire disease not only in tobacco but also in a large number of plant species representing many different families.[1] It has been suggested[2] that the toxin owes its biological activity to its chemical affinity for the chlorophyll molecule.

Materials and Methods.—In studying the mode of action of the wildfire toxin the unicellular plant *Chlorella vulgaris* was selected as the test object. Chlorella develops well on a simple readily reproducible medium of the following composition: KNO_3 1.5 g., $Ca(NO_3)_2 \cdot 4H_2O$ 1.5 g., $MgSO_4 \cdot 7H_2O$ 2.4 g., KH_2PO_4 2.4 g., $FeSO_4$ 0.02 g., $MnSO_4 \cdot 4H_2O$ 0.001 g., H_3BO_4

0.001 g., CuSO$_4$ 0.0001 g., ZnSO$_4\cdot$7H$_2$O 0.0001 g., sucrose 20 g., distilled H$_2$O 1000 cc.

In these studies the cultures were grown in 50-cc. Erlenmeyer flasks. The basic medium together with supplementary substances totaling 10 cc. were placed in each flask. Sterilization was accomplished in an autoclave at 10 pounds' pressure for 10 minutes. Heat-labile compounds as well as the wildfire toxin were sterilized by filtration through Jena sintered glass
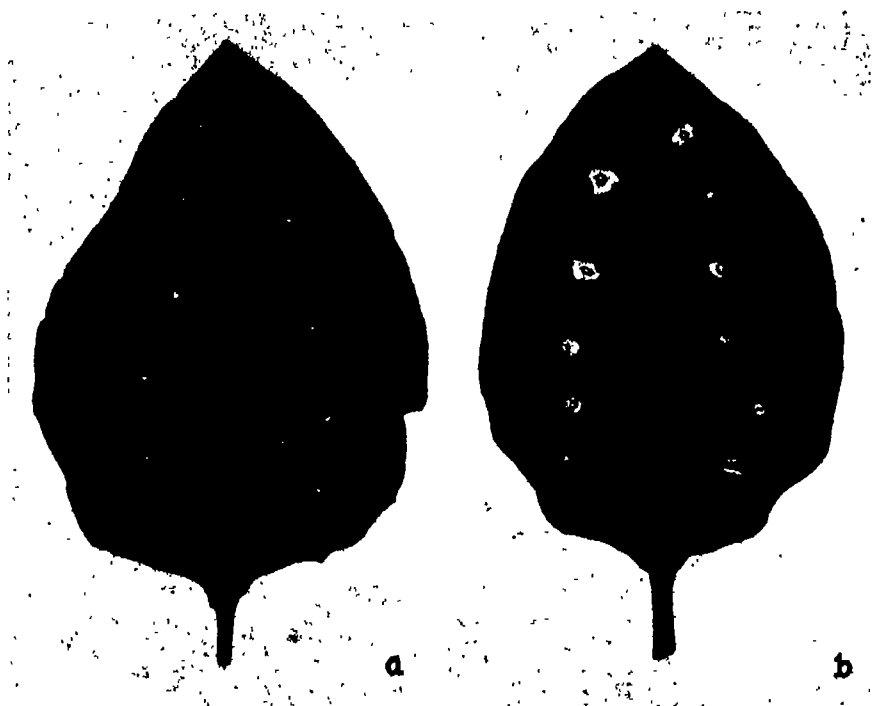
FIGURE 1

Tobacco leaves inoculated with (*a*) sterile wildfire toxin, (*b*) methionine sulfoximine. Note the similarity of chlorotic halos produced around the points of inoculation by the two substances.

bacteriological filters, porosity G 5 on 3. These compounds were added aseptically to the basic medium at the desired concentration. The flasks were inoculated with one drop of a one-week-old culture of Chlorella. Cultures were permitted to develop in an incubator at 26°C. for 5 days unless otherwise stated.

The partially purified toxin used in these experiments was prepared in the following manner. The bacteria were grown in large flasks containing a

solution of mineral salts and sucrose for 4 days at 25°C. The medium was well aerated during the incubation period. Following incubation the organisms were removed from the solution by centrifugation and the filtrate was treated with norite A at a pH of about 6.5. The adsorbed toxin was eluted with methanol and the methanol was removed by distillation under vacuum at a temperature not exceeding 35°C. The resulting residue was taken up in the medium used for growing Chlorella. The solution was standardized by adding enough basic media so that when assayed on tobacco leaves the toxin-containing solution produced a halo 5 mm. in diameter at a dilution of 1–100. Sterilization of this preparation was accomplished by filtration through a sintered glass filter. The stock toxin solution which was stored at 4°C. was used for all experiments reported here.

Experimental Results.—When growth of Chlorella in a medium containing dilute wildfire toxin (the medium gave a faint halo at a dilution of 1–5 when tested on tobacco) was compared with growth in a similar but non-toxin-containing medium, it was found that multiplication of the organism, although not completely inhibited, was only about one-third as rapid in the former as it was in the control medium after a 12-day incubation period. High concentrations of toxin completely inhibited growth of Chlorella. These results suggested that the toxin either interfered with synthesis of some essential growth factor or impaired the ability of Chlorella to utilize such a factor. In testing these possibilities it was found that when a medium containing toxin in a concentration sufficient to inhibit completely the growth of Chlorella (the medium gave a halo 5 mm. in diameter at a dilution of 1–50 when tested on tobacco leaves) was supplemented with 0.5 per cent liver extract, the deleterious effect of the toxin on the growth of the organism was completely negated. The toxin itself was not inactivated by the liver extract. The liver extract, therefore, contained a factor or factors necessary for the growth of Chlorella that were rendered unavailable for normal growth of that organism as a result of the action of the toxin.

A large number of compounds, among which were included the water-soluble vitamins, the amino acids, representative purine and pyrimidine derivatives, certain sulfhydryl compounds, as well as other substances known to be present in liver extract, were tested individually in an attempt to find the factor present in the liver extract capable of overcoming the toxic influence. Of these only one, *dl*-methionine, was capable of completely neutralizing the deleterious effect of the toxin on the growth of Chlorella. Growth of Chlorella in a toxin-containing medium supplemented with 0.1 mg./cc. of *dl*-methionine was comparable in every respect to the growth of the organism on a non-toxin-containing control medium. The natural form of methionine was found to be active, while the *d* isomer was entirely without effect. Certain other amino acids, such as *dl*-leucine, *dl*-norleucine and to a lesser extent *l*+arginine, *dl*-phenylalanine and *l*-tyrosine, were partially

effective in neutralizing the toxic influence when relatively small concentrations of toxin were present in the culture medium.

The probable pathway of methionine synthesis has been established for a number of different organisms. The following scheme with minor variations, cysteine → cystathionine → homocysteine → methionine, appears to hold for *Bacillus subtilis*,[4] *Escherichia coli*,[5,6] and Neurospora.[7] When tested separately, however, *l*-cystathionine and *dl*-homocysteine were found to be ineffective in overcoming the toxic influence on the growth of Chlorella. *l* + cysteine was found to be quite toxic for the organism.

If the synthesis of methionine in Chlorella follows a pathway similar to that reported for certain other organisms, then it would appear that either (1) a metabolic block occurs in the final step in the synthesis of methionine, possibly by virtue of the fact that the toxin prevents the methylation of homocysteine, or (2) that *l*-methionine is being synthesized by the organism in the presence of the toxin but that the toxin prevents the normal utilization of that compound. In order to determine which of the two possibilities was involved here, the growth of Chlorella in three different concentrations of toxin was tested against a range of concentrations of *l*-methionine. In this experiment the toxin-containing media, when assayed on tobacco leaves, produced halos 5 mm. in diameter at a dilution of 1–50, 1–25 and 1–10, respectively. When the data obtained following a 3-day incubation period were plotted it was found that the resulting curves were characterized over a range of concentrations by a more or less constant ratio between metabolite and inhibitor. Partially purified toxin was used in these experiments, however, and the final interpretation of results obtained in this phase of the work is withheld until the experiments can be repeated with chemically pure toxin. The results obtained with the impure preparation nevertheless suggest that the toxin-methionine antagonism is competitive, indicating that *l*-methionine is being synthesized by Chlorella in the presence of the toxin but that normal utilization of that compound is impaired by the action of the toxin.

While it has been possible to reverse completely the deleterious effect of the toxin on the growth of Chlorella with *l*-methionine, a similar reversal has not yet been possible when tobacco leaves containing chlorotic halos were immersed in a solution of that compound. That the mechanism of action is similar in both organisms, however, is suggested by the fact that the known methionine antagonist, methionine sulfoximine, in a concentration of 0.1 mg./cc. produced chlorotic halos in tobacco leaves that were indistinguishable from those produced by the wildfire toxin, as shown in Figure 1*b*.

Summary and Conclusions.—The bacterial toxin associated with the wildfire disease of tobacco has been shown to exert its biological effect by interrupting the methionine metabolism of plant cells.

The inhibitory influence of the toxin on the growth of Chlorella was completely reversible in the presence of *l*-methionine. A similar reversal has not yet been accomplished in tobacco. That the mechanism of action is similar in both organisms, however, is suggested by the fact that methionine sulfoximine, a known methionine antagonist, produced chlorotic lesions in tobacco leaves that were indistinguishable from those produced by the bacterial toxin.

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THE GROWTH-PROMOTING PROPERTIES OF QUINIC ACID*

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Evidence is accumulating that there is a common metabolic precursor to many of the benzene ring derivatives found in living organisms[1–4]. Recent work by Davis[3] and Tatum[4] indicates that one such precursor is the naturally occurring shikimic acid (Fig. 1) since this compound serves as a growth factor for certain mutants of *Escherichia coli*[3] and Neurospora[4] which otherwise require a combination of tyrosine, phenylalanine, tryptophan and *p*-aminobenzoic acid for growth. These mutants cannot utilize the closely related, naturally occurring quinic acid (Fig. 1) as a substitute for any of their requirements.[5, 6]

In an earlier investigation of quinic acid and shikimic acid, Fischer and Dangschat[5, 6] showed that quinic acid is chemically convertible to shikimic

acid and that the latter is convertible to glucodesonic acid. They pointed to the possibility that these compounds are formed directly from glucose.

For these reasons the growth-promoting properties of quinic acid and shikimic acid were reinvestigated with a Neurospora mutant, C-86, which is capable of utilizing a variety of aromatic compounds for growth[2, 7, 8]. Table 1 gives a list of compounds utilized by C-86 together with those utilized by several other mutants used in this investigation[2, 7-9].

Experimental.—Methods: The growth responses of the several strains of Neurospora tested were measured as dry weight of mycelium after 120 hours at 25° in 20 ml. of the standard Fries medium, adjusted to pH 4.6. Inoculations were made with drops of suspensions of conidia in sterile water. The shikimic acid[10] and the quinic acid[11] were filter sterilized and added sterilely to the autoclaved medium after cooling.
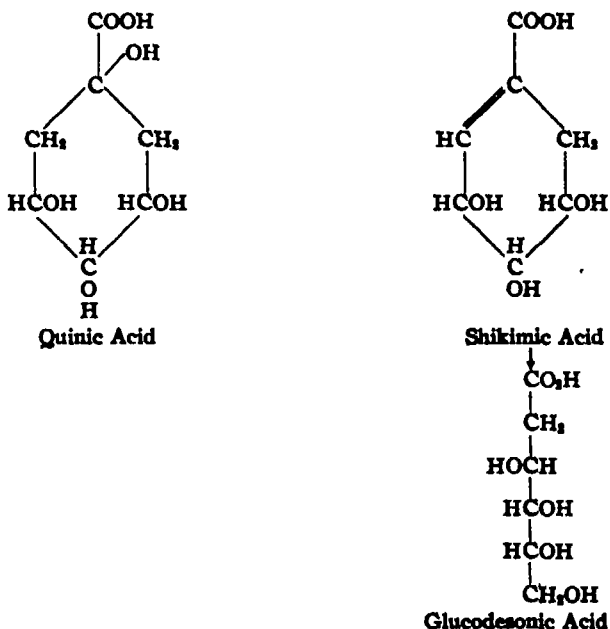
FIGURE 1

Analytical: The quinic acid used was recrystallized from water, melted at 162–163° and had a molecular rotation of −42.8° at 25°. Calculated, C, 43.75, H, 6.29; found, C, 43.49, H, 6.18.

Responses of Mutant Strains: The following compounds were tested for their ability to promote the growth of Neurospora, strain C-86: shikimic acid, quinic acid, dopa, protocatechuic acid, *p*-aminobenzoic acid and gentisic acid. Except for a very small response to gentisic acid, quinic acid

is the only one of these which supports the growth of C-86. Table 2 compares this effect with the growth-promoting effect of tryptophan.

While the activity of quinic acid is about the same as that reported for tyrosine and *trans*-cinnamic acid at low levels,[2] the inhibitory effect noted at higher concentration with *trans*-cinnamic acid is not observed with quinic acid.[11]

To eliminate the possibility that the growth response of C-86 to quinic acid is due to contamination with small amounts of other compounds which promote its growth (table 1), the same sample of quinic acid was filter sterilized and tested at 1- and 5-mg. levels against each of the other mutants listed in table 1. None grew at either concentration of quinic acid.

TABLE 1

COMPOUNDS UTILIZED BY VARIOUS NEUROSPORA MUTANTS

STRAIN	TYROSINE OR *trans*-CINNAMIC ACID	PHENYL-ALANINE	ANTHRA-NILIC ACID	INDOLE	TRYPTO-PHAN	KYNURE-NINE	3-OH-KYNURE-NINE	3-OH-ANTHRA-NILIC ACID	NICO-TINIC ACID
C-86	+	+	+	+	+	+	+	+	+
E-5212	−	+	−	−	−	−	−	−	−
B-1312	−	−	+	+	+	+	−	−	−
39401	−	−	−	+	+	+	+	+	+
10575	−	−	−	+	+	−	−	−	−
C-83	−	−	−	−	+	−	−	−	−
E-5029	−	−	−	−	−	−	+	+	+
4540	−	−	−	−	−	−	−	−	+

TABLE 2

GROWTH OF NEUROSPORA MUTANT C-86 IN THE PRESENCE OF (−)-QUINIC ACID AND L-TRYPTOPHAN

γ	DRY WT. OF MOLD, MG.	
	L-TRYPTOPHAN	(−)-QUINIC ACID
25	15 8	...
50	22 8	...
100	33.6	...
250	...	2.0
500	69.2	7.6
1000	...	21.8
3000	...	38.4
5000	...	48.2

Discussion.—These experiments demonstrate three main differences between the shikimic acid and quinic acid mutants of Neurospora.

(1) The mutant which utilizes shikimic acid cannot utilize quinic acid and vice versa.

(2) C-86 grows in the presence of any one of a number of compounds which presumably are related metabolically. The shikimic acid mutant requires four aromatic compounds for growth in the absence of shikimic acid.

(3)   *p*-Aminobenzoic acid has no effect on C-86, while it is an absolute requirement for the shikimic acid mutant in the absence of shikimic acid.

The apparent lack of correlation between the results obtained with C-86 and the shikimic acid mutant can be explained by one of several schemes. However, the data presently available do not warrant extensive speculation about the presumed metabolic relationship between quinic acid and shikimic acid, between them and glucose or between them and the aromatic compounds they replace. The evidence would nevertheless seem to favor the view that at least several of the aromatic compounds found in Neurospora arise from a common non-aromatic, cyclical intermediate.

Summary.—Quinic acid promotes the growth of Neurospora, strain C-86, which otherwise requires for growth any one of several aromatic compounds. The closely related shikimic acid is completely without activity for this strain.

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¹⁰ Furnished by Dr. Roger Stanier, University of California.

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FURTHER STUDIES ON THE PURINE AND PYRIMIDINE METABOLISM OF TETRAHYMENA*

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Introduction.—It has been shown that the animal microorganism, Tetrahymena, possesses a pattern for the metabolism of purines and pyrimidines which is at variance with other animals so far critically studied. Unlike higher animals, Tetrahymena has lost capacities for the synthesis of certain purines and pyrimidines. Thus it was shown[1] that of the naturally occurring purines only guanine would satisfy its requirement. Adenine and hypoxanthine are metabolized, however, since both show guanine sparing action. Xanthine was found to be inactive. It was likewise shown that only uracil, of the naturally occurring pyrimidines, would satisfy the pyrimidine requirement of Tetrahymena. Unlike the purine specificity, however, a block occurs which makes it impossible for the organism to carry out the riboside linkage with cytosine, for either cytidine or cytidylic acid will replace uracil, uridine or uridylic acid.

By the use of a number of substituted purines[2] and pyrimidines,[3] it was possible to gain some information as to the specific metabolic abilities of this organism regarding these important classes of compounds. It was shown, for instance, that of the methyl-substituted xanthines all were inert or inhibitory with the single exception of 1-methyl xanthine. This latter compound would replace guanine with 15 per cent activity. Methyl substitutions on the guanine molecule reduced its activity for guanine replacement. 1-Methylguanine was 75 per cent as effective as guanine, and 7-methylguanine and 1,7-dimethylguanine were weakly active in sparing guanine. Any substitution on the uracil molecule reduced the activity to 1 per cent or less.

Inasmuch as the metabolic pathways for nucleic acid constitutents have assumed considerable importance, it is the purpose of this paper to report extensions of our previous findings and to compare briefly the biosynthetic abilities of Tetrahymena with those of other organisms.

Experimental.—*Tetrahymena geleii* W, grown in pure (bacteria-free) cultures, was used throughout this work. The basal medium used is given in table 1. A new technique using the Brewer Automatic Pipetting Machine, described elsewhere,[4] was used for accuracy and speed. Quantitative growth determinations were made turbidimetrically by use of a Lumetron photoelectric colorimeter with a red (650) filter. The tubes were incubated at 25°C. for 96 hours in a slanted position for adequate aeration.[5] All

series were run in triplicate and the experiments repeated varying numbers of times.

Results.—The imidazole ring: It was earlier reported[6] that Tetrahymena was able to synthesize guanine from 2,4-diamino-5-formylamino-6-hydroxy pyrimidine. The latter compound was said to possess 13.5 per cent activity when compared to guanine hydrochloride dihydrate on a weight basis.

TABLE 1

BASAL MEDIUM[a]

DL-alanine	110	$MgSO_4 \cdot 7H_2O$	100.00
L-arginine HCl	206	$Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$	25.00
L-aspartic acid	122	$FeCl_3 \cdot 6H_2O$	1.25
Glycine	10	$MnCl_2 \cdot 4H_2O$	0.50
L-Glutamic acid	233	$ZnCl_2$	0.05
L-histidine HCl	87	$CaCl_2 \cdot 2H_2O$	50.00
DL-isoleucine	276	$CuCl_2 \cdot 2H_2O$	5.00
L-leucine	344	K_2HPO_4	1000.00
L-lysine HCl	272	KH_2PO_4	1000.00
DL-methionine	248	Dextrose	2500.00
L-phenylalanine	160	Na acetate	1000.00
L-proline	250	Tween 85[b]	700.00
DL-serine	394	Guanylic acid[c]	30.00
DL-threonine	376	Adenylic acid[c]	20.00
L-tryptophane	72	Cytidylic acid[c]	25.00
DL-valine	162	Uracil[c]	10.00
Ca pantothenate	0.10	Protogen[d]	1 unit
Nicotinamide	0.10		
Pyridoxine HCl	1.00		
Pyridoxal HCl	0.10		
Pyridoxamine HCl	0.10		
Riboflavin	0.10		
Pteroylglutamic acid	0.01		
Thiamine HCl	1.00		
Biotin (free acid)	0.0005		
Choline Cl	1.00		

[a] All amounts are given in micrograms per ml. of final medium.
[b] Atlas Powder Company.
[c] Omitted in appropriate experiments.
[d] Furnished by the Lederle Research Laboratories through the courtesy of Dr. E. L. R. Stokstad.

Since that report, different samples of the formylamino compound have been used, and it has been found that the original sample contained approximately 9 per cent guanine (on a molar basis) as a contaminant. The presence of guanine was shown by the fact that the activity for Tetrahymena was not diminished by a hydrolysis which completely destroyed the formylamino compound. The absence of the formylamino compound in hydrolyzed samples was demonstrated by ultra-violet absorption measure-

ments. Other samples of the formylamino pyrimidine, free of guanine contamination, had no activity for Tetrahymena either before or after hydrolysis.   It is apparent, therefore, that this organism does not possess the necessary enzymes for the dehydration of 2,4-diamino-5-formylamino-6-hydroxy pyrimidine and is incapable of forming the imidazole ring. As might be expected, 2,4-diamino-5-acetylamino-6-hydroxy pyrimidine and 2,4-diamino-5-propionylamino-6-hydroxy pyrimidine were inert, as was 2,4,5-triamino-6-hydroxy pyrimidine.

Tetrahymena is able to demethylate position 8 of guanine to some extent (8-methyl guanine was 5 per cent as active as guanine), but 8-ethyl guanine[7] is inert.
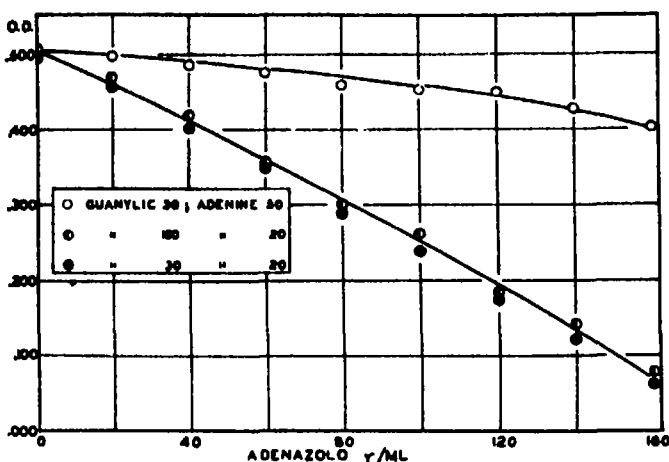
FIGURE 1

Release of the inhibition caused by 7-amino-1-v-triazolo(d)-pyrimidine (adenazolo) by adenine, but not by guanylic acid. The amounts of guanylic acid and adenine are in γ/ml.   Guanine and hypoxanthine, like guanylic acid, do not release.

Triazolo analogs: It was previously reported[2] that the triazolo analog of hypoxanthine (7-hydroxy-1-v-triazolo(d)pyrimidine) was not inhibitory to Tetrahymena within the ranges tested.   This finding, when considered in relation to the activity of the adenine analog (7-amino-1-v-triazolo(d)-pyrimidine) to be considered below, assumes importance in evaluating the metabolism of hypoxanthine and adenine by this organism.

The adenine analog, which we shall call adenazolo in keeping with the previous nomenclature of these compounds,[8] is an active purine inhibitor with an inhibition index (half maximum) of 5.   This inhibition is specifically reversed by adenine. Guanine and hypoxanthine are inactive. A typical experiment is illustrated in Figure 1 showing the inability of guanine to re-

verse the adenazolo inhibition. It is to be remembered that both hypo-xanthine and adenine are active in sparing guanine, and it was suggested[2] that hypoxanthine might be used by Tetrahymena for the synthesis of ino-sinic acid. It now appears more likely that the sparing action of hypoxan-thine is due to its ready amination to adenine; when this system is blocked (by hypoxanthazolo), it is of little importance and no effect is noted.
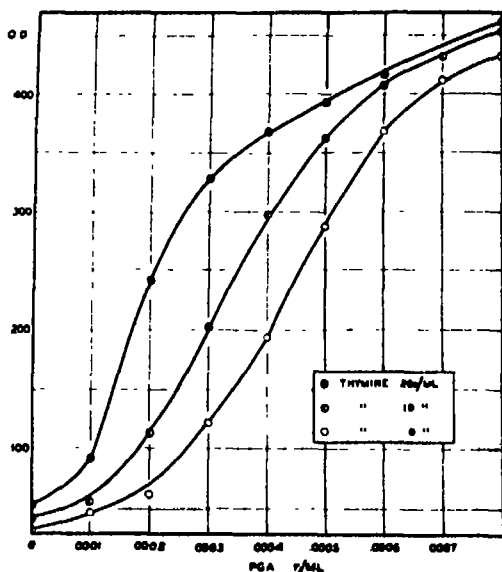
FIGURE 2

Dose response to pteroylglutamic acid (PGA) in media with and without thymine. The basal medium contained the following nucleic acid com-ponents in γ/ml.: guanylic acid, 30; adenylic acid, 20; cytidylic acid, 25; uracil, 10. Under the conditions of these experiments half maximum growth in the medium devoid of thymine required approximately 0.00046 γ/ml. of PGA; with 10 γ/ml. of thymine this requirement was reduced to 0.00035 γ/ml. and with 20 γ/ml. of thymine the requirement was further reduced to 0.00021 γ/ml. Raising the level of thymine up to 50 γ/ml. re-sulted in no greater sparing action than 20 γ/ml. of thymine.

Pyrimidines. Orotic acid: Inasmuch as orotic acid (4-carboxyuracil) has been found to have activity for certain pyrimidine-less Neurospora mutants[9, 10] and has been found to be incor-porated, in contrast to uracil, in mammalian nucleic acid,[11, 12] it was im-portant to test the activity of this compound for Tetra-hymena. It was found to be entirely inactive for this organism, neither replacing nor sparing uracil.[13] This indicates that Tetrahymena does not possess the specific decarboxylase necessary for the formation of uracil from orotic acid. It cannot be shown, therefore, whether or not orotic nucleoside can be formed, as occurs in the mammal,[12] although this appears unlikely.

Thymine and thymidine: Our data indicate that thymine is not formed by the methylation of uracil but is synthesized by Tetrahymena from non-pyrimidine precursors. It was earlier reported[2] that neither thymine nor thymidine could replace uracil, and critical quantitative tests now show that neither has any sparing action. This would not be true if some uracil or uridine were being used for their synthesis. Moreover thymine is active in sparing pteroylglutamic acid (Fig. 2) which is taken to mean that PGA

is active in one or more steps in the synthesis of thymine. Thymidine[14] is approximately twice as active as thymine as a sparer of PGA, indicating that PGA may function also in performing the desoxyriboside linkage. These observations are in agreement with those on *Lactobacillus casei*[14, 16] and *Streptococcus fecalis*[16, 17] where thymine was found capable of replacing PGA. Tetrahymena differs from these bacteria, however, in that thymine and thymidine will only spare, never replace, PGA. One or more vital functions in the metabolism of this animal, in addition to those concerned with thymine and its desoxyriboside, are PGA controlled.

Discussion.—We can now reconstruct a probable course of metabolic pathways in Tetrahymena for purines and pyrimidines and compare these to what has been learned about these compounds in other organisms.
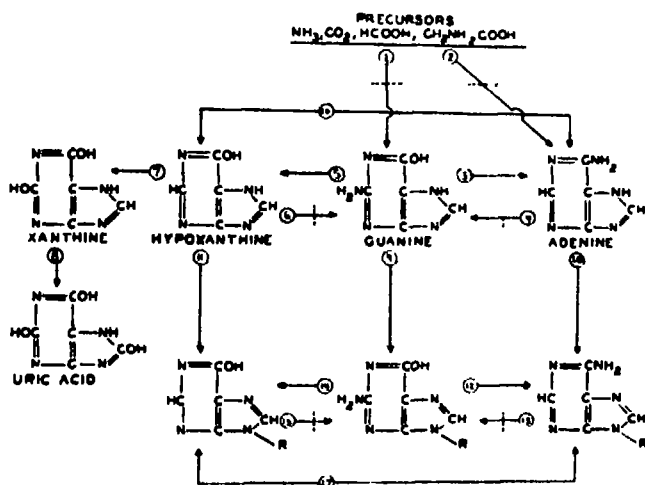
FIGURE 3

Schematic representation of the metabolism of purines. The dotted lines represent blocks in specific enzyme systems which occur in Tetrahymena. For evidence see text.

Figure 3 is a summary of the various steps in the metabolism of purines. The dotted cross lines on the arrows refer to blocks in the enzyme systems of Tetrahymena.

Reactions 1 and/or 2 are known to occur in the rat and pigeon, where it has been shown by tracer techniques that the purine ring is built up as follows: nitrogens 1, 3 and 9 come from the ammonia pool;[18] carbons 2 and 8 are derived from formate;[19-21] carbon 6 comes from CO_2[20, 21] and carbons 4 and 5 and nitrogen 7 come from glycine.[20, 21] Steps of this nature (not necessarily from the same precursors) must occur in all organisms without an exogenous purine requirement. That these reactions are blocked in

Tetrahymena is shown by the requirement for guanine and the sparing action of adenine. Reaction 3 probably takes place in Tetrahymena. It may also take place in the four guanine-less mutants of the ascomycete Ophiostoma reported by Fries.[22,23] This reaction is blocked in the rat, as it was found that the administration of isotopically labeled guanine did not lead to the appearance of the isotope in the tissue purines.[24,25] Reaction 4 is blocked in Tetrahymena but may take place in the rat. Reaction 5 may take place in Tetrahymena but probably does not take place in the mammal, for if it did, in view of the apparent wide-spread ability of many or all organisms to aminate hypoxanthine to adenine, then dietary guanine would be converted to adenine via hypoxanthine. This appears to be precluded by the results of the tracer studies. Guanase activity occurs in the rat, but

FIGURE 4
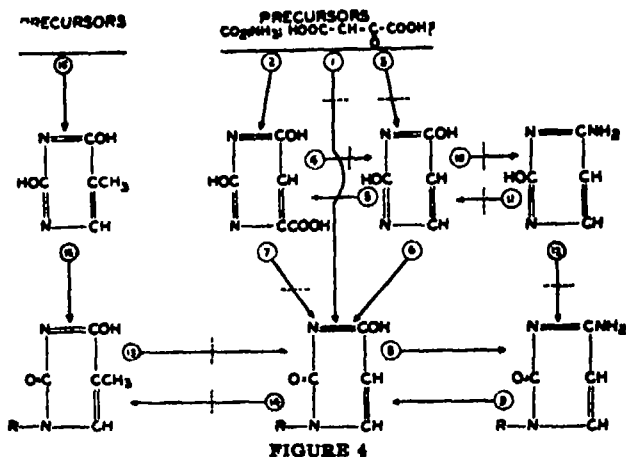
Schematic representation of the metabolism of pyrimidines. The dotted lines represent blocks in specific enzyme systems which occur in Tetrahymena. For evidence see text.

the result is xanthine, not hypoxanthine. Reaction 6 is blocked in Tetrahymena and the Ophiostoma mutants as evidenced by the fact that hypoxanthine will not replace guanine. Nothing is known about a xanthine oxidase (reactions 7 and 8) in Tetrahymena, although these are well-recognized reactions in many forms.

Reaction 9 is known to occur in Tetrahymena and the guanine-less Ophiotoma mutants[22,23] but it probably does not occur in the rat, while reactions 10 and 11 certainly do occur in the mammal and reaction 10, at least, occurs in Tetrahymena. Reaction 12 takes place in Tetrahymena, and may also take place in the rat, while reaction 13 is blocked in Tetrahymena as shown by the fact that adenosine and adenylic acid are incapable of replacing guanine while guanosine and guanylic acid are fully active.[1] Reac-

tion 14 is not known to occur in Tetrahymena, but, inasmuch as inosinic acid will not meet the guanine requirement, reaction 15 is certainly blocked. Reactions 16 and 17 are known to occur in many organisms and probably take place in Tetrahymena, as indicated by the different results obtained when hypoxanthine and adenine inhibitors were employed.

Figure 4 is a summary of the various steps in the metabolism of pyrimidines. Inasmuch as Mitchell, *et al.*,[9, 10] have shown that certain pyrimidineless Neurospora mutants can use non-pyrimidine compounds and have suggested that ring closure does not precede the riboside linkage, we may suppose that at least three alternate routes of pyrimidine nucleoside synthesis developed in pre-animal systems. The first (reaction 1) is illustrated by the Neurospora mutants,[9, 10] where it appears that oxaloacetic acid is converted to amino-fumaric acid and the riboside linkage completed before the pyrimidine ring is closed to form uridine. Whether these reactions are utilized to some degree in the rat is not known, but it is known that nitrogens 1 and 3 of the pyrimidine ring are derived from the ammonia pool[16] while carbon 2 is derived from CO_2.[21] It seems clear from the tracer studies of Arvidson, *et. al.*,[12] that orotic acid is utilized in the synthesis of uridine and is therefore synthesized from non-pyrimidine precursors (reaction 2). The interpretation placed upon these observations by Arvidson, *et al.*, was that orotic acid was linked with the sugar to form orotic nucleoside and then decarboxylated to uridine. Reactions 1 and 7 are blocked in Tetrahymena as is also probable for reaction 2. It is also clear that reaction 3 is blocked in this organism, which leaves preformed uracil as a required compound. Reaction 4 is blocked in Tetrahymena, while reaction 5 is blocked in the rat.[24] Reaction 6 is blocked in the rat,[24] but this reaction (sugar linkage to uracil) is very efficient in Tetrahymena, as evidenced by the fact that uracil and uridine are of approximately equal activity on a molar basis.[3] Reductive amination of uridine and oxidative deamination of cytidine (reactions 8 and 9) appear to be carried out with ease in many organisms[23, 27, 28] and Tetrahymena is no exception. Reactions 10, 11 and 12 are blocked in Tetrahymena as they appear to be likewise blocked in Neurospora mutants,[27, 28] Ophiostoma mutants[32, 33] and in the rat.[26] The fact that cytosine does not replace or spare uracil for Tetrahymena denotes blocks in reactions 11 and 12, while block 10 is inferred from the fact that uracil is as active as uridine on a molar basis. This would hardly be the case if part of the uracil were being sacrificed to produce an inert compound.

As has been stated, thymine is inactive for uracil replacement and sparing,[3] so there appears to be no methylation of uracil to thymine. Likewise thymidine will not replace nor spare uracil, which denotes blocks in reactions 13 and 14. Since large quantities of desoxyribose nucleic acid are present in these cells, it seems probable that thymine must be synthesized

from non-pyrimidine precursors (reactions 15 and 16). Both reactions 15 and 16 appear to depend upon pteroylgutamic acid, because thymine and thymidine are active in sparing this compound.

Recently Reichard[29] has presented evidence which indicates that thymine is derived from orotic acid in the rat. Administration of N^{15} labeled orotic acid resulted in the isotope appearing in low concentrations in the cytosine and thymine isolated from the tissue desoxyribose nucleic acid. If the purity of the isolated pyrimidines was satisfactory, this is a significant finding and would indicate that the rat has enzymes capable of methylating position 5 of orotic acid and/or orotic acid riboside and/or uridine.

Summary.—The animal microorganism, Tetrahymena, cannot synthesize the imidazole ring as evidenced by the failure of 2,4-diamino-5-formylamino-6-hydroxy pyrimidine to replace guanine. The triazolo analog of adenine (adenazolo) is inhibitory, with an inhibition index of 5. This inhibition is not released by any purine tested except adenine. Orotic acid is inactive for Tetrahymena, indicating a lack of specific decarboxylating enzymes. Evidence for the synthesis of thymine from non-pyrimidine precursors is presented. The synthesis of thymine and the formation of its desoxy-riboside appear to be PGA controlled. Thymidine is approximately twice as active in sparing PGA as is thymine. Comparative enzymatic capacities for purine and pyrimidine metabolism are discussed.

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PARTIAL ORDERING IN THEORY OF STOCHASTIC PROCESSES

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We take a directed set $\{\alpha\}$, so that $\alpha < \alpha$, $\alpha < \beta$ and $\beta < \gamma$ implies $\alpha < \gamma$, and to any α and β there is a γ such that $\alpha < \gamma$ and $\beta < \gamma$; and we consider a family of spaces $\{R_\alpha\}$ indexed by it, and for any $\alpha < \beta$ a map $x_\alpha = \varphi_{\alpha\beta} x_\beta$ from R_β onto all of R_α, also called projection, such that for $\alpha < \beta < \gamma$ we have $\varphi_{\alpha\beta}\varphi_{\beta\gamma} = \varphi_{\alpha\gamma}$ ("consistency"). In the direct product of all spaces R_α there is a "diagonal" set $\{x_\alpha\}$ in which $x_\alpha = \varphi_{\alpha\beta} x_\beta$ for $\alpha < \beta$ and this is the so-called projective limit of the given inverse mapping system.[1] The limit will be denoted by R or more explicitly by R_∞, and we note that each point x_α of every R_α is the α-th component of at least one point in R. The space could, but will not be appended to the given family by putting $\alpha < \infty$, $x_\alpha = \varphi_{\alpha\infty}^{-1}(\{x_\beta\})$.

We denote by A_α a set of subsets of each R_α, such that for $S_\alpha \in A_\alpha$ and $\alpha < \beta$ we have $\varphi_{\alpha\beta}^{-1}(S_\alpha) \in A_\beta$. Each $\varphi_{\alpha\infty}^{-1}(S_\alpha)$ is a subset of R and the set of the latter subsets will be denoted by A or A_∞. If each A_α is a Boolean algebra then so is A itself, but if they are σ-algebras then A need no longer be one. We assume that on each algebra A_α there is given a finitely additive measure m_α, so that $m_\alpha R_\alpha = 1$, and that they are consistent, $m_\beta[\varphi_{\alpha\beta}^{-1}(S_\alpha)] = m_\alpha S_\alpha$. This gives rise to a such-like measure mS on A, $m[\varphi_{\alpha\infty}^{-1}(S_\alpha)] = m_\alpha S_\alpha$, but σ-additivity need no longer reproduce itself. However the following theorem can be stated which for

ordinary sequences of Euclidean spaces was first given by A. Kolmogoroff,[2] and for directed sets of Euclidean spaces in a previous paper of ours.[3]

THEOREM 1. *If each R_α is a Hausdorff space, and the given σ-measures m_α are such that for any S_α and any $\epsilon > 0$ there exists a compact subset F_α of S_α such that $m_\alpha F_\alpha > m_\alpha S_\alpha - \epsilon$; then the limit measure mS is σ-additive on a σ-closure of A.*

Proof: R itself is also a Hausdorff space,[1] and if F_α is compact in R_α then $\varphi_{\alpha\infty}{}^{-1}(F_\alpha)$ is compact in R. Therefore, to any S in A and any $\epsilon > 0$ there exists a compact subset F of S such that $mF > mS - \epsilon$.

Now, by a general criterion, a finitely additive measure in S has a σ-extension if $S^1 \supset S^2 \supset \ldots \rightarrow 0$ implies $mS^n \rightarrow 0$. If it is not fulfilled, there exists a sequence $\{S^n\}$, with $\lim S^n = 0$ and $mS^n \geq 2m_0 > 0$. But then we could find compact subsets F^n of S^n such that $m(S^n - F^n) \leq m_0 2^{-n}$. The intersections $G^n = \Pi_{j=1}^n F^j$ would still be compact subsets of S^n, and $m(S^n - G^n) \leq m_0$. Therefore $mG^n \geq m_0$, and no G^n is empty. But $G^n \supset G^{n+1}$, and thus the set-limit of G^n could not be empty, contrary to the tentative assumption that the set limit of S^n so was. q. e. d.

As an application consider on any space M an arbitrary (non-continuous) path $x(t)$, $a < t < b$, a and b fixed finite or infinite. The index α shall be any selection of time points $\alpha = (t_0, t_1, \ldots, t_n)$, $t_0 < t_1 < \ldots < t_n$; and if $\beta = (\tau_0, \tau_1, \ldots, \tau_m)$ then put $\alpha < \beta$ if and only if $n \leq m$ and each t_ν equals some τ_μ. For given α the space R_α shall be the $(n + 1)$-fold product $Mx \ldots xM$, A_α shall be a suitable algebra of subsets, and $m_\alpha(S_\alpha)$, $S_\alpha \epsilon A_\alpha$, shall be an *a priori* probability for the $n + 1$ points $y(t_0), \ldots, y(t_n)$ on the arbitrary path in M to be lying in S_α. And these *a priori* probabilities shall be "consistent" with each other in the manner previously stipulated. The conclusion from Theorem 1 is then as follows. If M has a topological Hausdorff structure, if we transmit this structure to R_α, and if the measurable sets S_α are Borel sets in the topology of R_α, then the assemblage of all arbitrary paths in M can be made into a stochastic process by introducing into it a probability measure of which the given *a priori* probabilities are the natural projections.

If we put $t_0 = $ constant, and correspondingly $x(t_0) = $ constant (all paths emanating from the same point), then R_α is an n-fold manifold, the theorem again applies, and the resulting stochastic process is a so-called Brownian motion. Furthermore, if we consider discrete time-points, $t_n = nh$, it suffices to consider indices α with only consecutive time elements, $\alpha = (0, h, 2h, \ldots, nh)$, and if the space M is the real line in the natural topology (or perhaps the complex plane) then the set-up is the classical case of a sequence of random variables $(x_0), x_1, x_2, \ldots$; and if there are given consistent joint distributions for say $x_1, \ldots, x_n$, $n = 1, 2, 3, \ldots$ then by Theorem 1 the infinite sequence can be made into a stochastic process, and this is the known theorem of Cantelli and Kolmogoroff.

This Euclidean situation was treated previously in a rather comprehensive manner.[3]

Next, consider on M a (finite or infinite) Lebesgue volume in which Borel sets are measurable, and relative to it in $0 < t < \infty$ a Markoff density $f(r, x; s, y)$, $r < s$, with the known properties

$$\int_M f(r, x; s, y)dv_y = 1, \qquad f \geq 0$$
$$\int_M f(r, x; s, y)f(s, y; t, z)dv_y = f(r, x; t, z).[*]$$

It gives rise to a Brownian motion as previously introduced if we define $m_\alpha S_\alpha$ as .

$$\int \ldots \int_S f(t_0, x_0; t_1, x_1)f(t_1, x_1; t_2, x_2)\ldots f(t_{n-1}, x_{n-1}; t_n, x_n)dv_{x_1} \ldots dv_{x_n},$$

and if the Markoff chain is stationary, that is, $f(r, x; s, y) = f(s - r; x, y)$, then on introducing the lengths $r_1 = t_1 - t_0$, $r_2 = t_2 - t_1$, ..., this $m_\alpha S_\alpha$ is

$$\int \ldots \int_S f(r_1; x_0, x_1)f(r_2; x_1, x_2)\ldots f(r_n; x_{n-1}, x_n)dv_{x_1}\ldots dv_{x_n}. \qquad (1)$$

This stochastic process is built up of infinitesimal transitions which are stochastically independent, but we can relax this restrictive independence by the following device.

Take a set-up $\{R_\alpha; A_\alpha\}$ as before, and on it not only one (consistent) *a priori* probability $m_\alpha S_\alpha$ but a whole family of such probabilities $m_\alpha^{(\rho)} S_\alpha$. Then any linear combination

$$m_\alpha S_\alpha = \sum_\rho \gamma_\rho m_\alpha^{(\rho)} S_\alpha$$

with $\gamma_\rho \geq 0$, $\sum_\rho \gamma_\rho = 1$, and suitable limits of such combinations, are again admissible probabilities. In particular, if we are given a stationary Markoff chain $f(r; x, y)$ then for fixed $t > 0$, $f(tr; x, y)$ is again such a chain, and by the rule just stated we obtain a "brownian motion" if in equation (1) we replace the integrand by the more general integrand

$$\int_{+0}^{\infty} f(tr_1; x_0, x_1)f(tr_2; x_1, x_2)\ldots f(tr_n; x_{n-1}, x_n)d\,\gamma(t)$$

where $\gamma(t)$ is monotone in $0 < t < \infty$ with $\gamma(+0) = 0$, $\gamma(\infty) = 1$.

This modification of one stochastic process into another by means of a positive integral transformation must be carefully distinguished from another one previously given[4] in which one Markoff chain is being modified into another Markoff chain by the following general procedure. Consider on M a stationary Markoff chain with a density $f(r; x, y)$, but assume that the time points $r, s, t, \ldots$ are elements of an arbitrary semigroup T in which a commutative associative addition $r + s$ is defined. We take another such general semigroup U with elements $u, v, w, \ldots$, and we assume that for $r \in T$, $u \in U$ we are given a real-valued function $\gamma(t; u)$ which is monotonely increasing on T, that is $\gamma(r + s; u) \geq \gamma(r; u)$, in

conjunction with a certain topology on T, so that for bounded continuous functions $h(r)$ and every u a Stieltjes integral

$$\int_T h(r) \, d \, \gamma(r; u)$$

shall be definable, and we assume that $\int_T d \, \gamma(r; u) = 1$ and

$$\int_{r + s = t} d \, \gamma(r; u) \, d \, \gamma(s; v) = d \, \gamma(t; u + v).$$

If now we introduce the function

$$g(u; x, y) = \int_T f(r; x, y) \, d \, \gamma(r; u) \tag{2}$$

then it has again all properties of a stationary Markoff chain and this is the transformation we wanted to describe. In particular if both T and U are the half line $(0, \infty)$ as usual, then a "matrix" $\{d \, \gamma(r, u)\}$ with the stated properties will be obtained from the Laplace Stieltjes expansion

$$\exp\left[-u\Phi(\lambda)\right] = \int_0^\infty e^{-\lambda t} d \, \gamma(t; u)$$

if $\Phi(\lambda)$ is any continuous function in $0 \leq \lambda < \infty$ with $\Phi(0) = 0$ for which such an expansion is available for $u > 0$. For instance, $\Phi(\lambda) = \lambda^\rho$, $0 < \rho < 1$, is of this kind.

But now take a set of functions $\Phi_p(\lambda)$, $p = 1, 2, \ldots$, of the kind just introduced, and for fixed k associate with them linear forms $L_p(u) = \gamma_{p1}u_1 + \ldots + \gamma_{pk}u_k$ with real coefficients γ_{pj}. If now we introduce in the space of k-dimensional vectors $u = (u_1, \ldots, u_k)$ the convex cone U which is defined by $L_p(u) > 0$, $p = 1, 2, \ldots$, then for u in U we again have an expansion

$$\exp\left[-\sum_p L_p(u)\Phi_p(\gamma)\right] = \int_0^\infty e^{-\lambda t} d \, \gamma(t; u).$$

If we take any unitemporal chain $f(r; x, y)$ and make the transformation (2) then the resulting process $g(r; x, y)$ is more or less what P. Lévy has been lately calling a multiple Markoff chain. If in particular we start with

$$f(r; x, y) = \sum_n e^{-r\lambda_n} \varphi_n(x)\varphi_n(y)$$

where $\{\lambda_n; \varphi_n\}$ are the eigenvalues and the eigenfunctions of a Laplacean $-\Delta$ on a compact Riemann space[4] then we have

$$g(u; x, y) = \sum_n \exp\left[-\sum_p L_p(u)\Phi_p(\lambda_n)\right] \varphi_n(x)\varphi_n(y),$$

and if we put $\sum_p L_p(u)\Phi_p(\lambda) = \sum_{j=1}^k u_j\Psi_j(\lambda)$, that is $\Psi_j(\lambda) = \sum_p \gamma_{pj}\Phi_p(\lambda)$, then the system of diffusion equations

$$-\frac{\partial g(u; x, y)}{\partial u_j} = \Psi_j(-\Delta_x)g(u; x, y), \qquad j = 1, \ldots, k$$

is satisfied, and this is a generalization of the Fokker-Planck equations

from unitemporal to multitemporal processes. A significant interpretation of multitemporal processes from the vantage point of such systems of equations would be indicated.

[1] See Lefschetz, S., *Algebraic Topology*, 1942, p. 31; and Braconnier, Jean, "Spectres d'espaces et de groupes topologiques," *Portugaliae Math.*, 1, 93–111 (1948).

[2] Grundbegriffe der Wahrscheinlichkeitsrechnung, *Ergebnisse der Mathematik*, 2, 196–262 (1933).

[3] "Stochastic Processes," *Ann. Math.*, 48, 1014–1061 (1947).

[4] "Diffusion Equation and Stochastic Processes," these PROCEEDINGS, 35, 368–370 (1949).

COHOMOLOGY THEORY OF ABELIAN GROUPS AND HOMOTOPY THEORY I

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If a topological space X is aspherical (i.e., if all homotopy groups other than the fundamental group vanish), Hurewicz has shown that the fundamental group π_1 of the space determines all the homology and cohomology groups of the space. After further investigations by H. Hopf, the authors and subsequently others[1] obtained an algebraic formulation for this determination, by exhibiting, for each abelian coefficient group G, a natural isomorphism

$$H^k(X; G) \cong H^k(\pi_1; G), \qquad k = 1, 2, \ldots \qquad (1)$$

between any cohomology group of the space and the corresponding (algebraic) cohomology group of the group π_1. The latter groups are defined as the cohomology groups of a certain cell complex $K = K(\pi_1, 1)$ depending only on the group π_1. The q-dimensional cells of this complex are all the q-tuples $[x_1, \ldots, x_q]$ of elements x_i of the group, and for $q > 1$ the boundary of any such cell is defined as

$$\partial[x_1, \ldots, x_q] = [x_2, \ldots, x_q] + \sum_{i=1}^{q-1} (-1)^i [x_1, \ldots, x_i x_{i+1}, \ldots, x_q] + $$
$$(-1)^q [x_1, \ldots, x_{q-1}]. \quad (2)$$

A "normalization" of this complex is also possible. If K_N is the subcomplex spanned by all cells $[x_1, \ldots, x_q]$ with some $x_i = 1$, then the cohomology groups $H^k(\pi_1; G)$ of K are isomorphic[2] to the relative groups of K modulo K_N.

In the study of the effect of higher homotopy groups upon cohomology (and homology) groups, it appears efficient to first isolate the effect of a single homotopy group. Hence, in this note we consider any arcwise connected topological space X, in which the mth (abelian) homotopy group $\Pi = \pi_m$ is given, with $m > 1$, and in which all the other homotopy groups vanish ($\pi_1 = 1$, $\pi_i = 0$ for $i \neq 1$, $i \neq m$). Previously, we obtained,[3] in analogy with (1), an expression

$$H^k(X; G) \cong H^k[K(\Pi, m); G] \qquad k = 1, 2, \ldots \qquad (3)$$

for the singular cohomology groups of the space in terms of the cohomology groups of a certain cell complex $K(\Pi, m)$ defined algebraically in a fashion similar to $K(\Pi, 1)$, but depending essentially upon the commutativity of Π (see the detailed description below).

The algebraic cohomology groups appearing in (1) and (3) are also essential for the definition[4] of certain "obstruction invariants" of spaces; these in turn, seem to be applicable to the problem of extension and classification of continuous mappings.[5] In a very few cases, the groups of $K(\Pi, m)$ have been computed[6] by applying deeper methods of homotopy theory to a suitably constructed topological space.

This note will state some of the results of a systematic study of the groups of $K(\Pi, m)$ by purely algebraic methods. A main result is the fact that these groups obey a curious analog of the Freudenthal suspension theorem.[7]

We begin with a description of the complex $K(\Pi, m)$. Choose for each positive integer q a standard q-dimensional simplex Δ_q with ordered vertices $(0, 1, \ldots, q)$, and let e_q^i, for $i = 0, 1, \ldots, q$, denote that mapping of Δ_{q-1} in Δ_q obtained by mapping the vertices $0, 1, \ldots, q - 1$ of Δ_{q-1} in order upon the vertices of Δ_q, omitting the vertex i of Δ_q. The q-cells of the complex $K(\Pi, m)$ are the m-dimensional cocycles $g \in Z^m(\Delta_q; \Pi)$; for each g, the mapping e_q^i yields a cocycle $F_i g = g e_q^i \in Z^m(\Delta_{q-1}; \Pi)$, and thus a $q - 1$ cell of K. The boundary of the q-cell g is defined as $\partial g = \sum (-1)^i F_i g$, where the addition from $i = 0$ to q is to be regarded as a sum of cells (and not as addition of cocycles). $K(\Pi, m)$ is the cell complex with these cells and this boundary formula.

The suspension homomorphism, mapping $K(\Pi, m)$ into $K(\Pi, m + 1)$, is obtained by first assigning, to each g, the "suspended" $(m + 1)$ cocycle Tg on Δ_{q+1}, defined for each $m + 1$ dimensional ordered simplex $(r_0, \ldots, r_{m+1})$ of Δ_{q+1} as

$$\begin{aligned}
(Tg)(r_0, \ldots, r_{m+1}) &= g(r_0, \ldots, r_m) && \text{if } r_{m+1} = q + 1, \\
&= 0 && \text{if } r_{m+1} < q + 1.
\end{aligned}$$

If g_0 denotes the cocycle which is identically zero, in the appropriate dimension, then the suspension mapping

$$Sg = Tg - g_0 \tag{4}$$

is a chain transformation (raising dimensions by 1) of $K(\Pi, m)$ into $K(\Pi, m + 1)$, and hence induces homomorphisms

$$S: H^{m+k}[K(\Pi, m + 1);\ G] \rightarrow H^{m-1+k}[K(\Pi, m);\ G] \tag{5}$$

on the corresponding cohomology groups, for $k = 1, 2, \ldots$.

THEOREM 1. *For $k \leq m$, the suspension homomorphism S is an isomorphism onto. For $k = m + 1$, it is an isomorphism into.*

The argument depends upon an algebraic reduction of the complex $K(\Pi, m)$ to a simpler "cubical" complex $Q(\Pi)$ depending on the abelian group Π alone. Each element x of Π determines a 1-cell $[x]$ of Q, each pair x, y, a 2-cell $[x, y]$ of Q, with boundary

$$\partial[x, y] = [x] + [y] - [x + y]. \tag{6}$$

A 3-cell of Q is a 2×2 square of elements in Π, with boundary

$$\partial\begin{bmatrix} x & y \\ r & s \end{bmatrix} = [x, y] + [r, s] - [x + r, y + s]$$
$$- [x, r] - [y, s] + [x + y, r + s]. \tag{7}$$

The proof that $\partial\partial = 0$ uses the hypothesis that Π is abelian.

In general, an $(n + 1)$-cell of Q will be a $2 \times 2 \times \ldots \times 2$ hypercube of dimension n, with entries in Π, and the boundary will consist of $3n$ terms formed, as in (7), by slicing the hypercube. Explicitly, label the vertices of the hypercube by the n-tuples $(\epsilon_1, \ldots, \epsilon_n)$ with each $\epsilon_i = 0$ or 1. An $(n + 1)$-cell of Q is any function σ with arguments all n-tuples $(\epsilon_1, \ldots, \epsilon_n)$ and with values in Π. The faces R_i, S_i, and P_i of σ are defined, for $i = 1, \ldots, n$, to be the n-cells

$$(R_i\sigma)(\epsilon_1, \ldots, \epsilon_{n-1}) = \sigma(\epsilon_1, \ldots, \epsilon_{i-1}, 0, \epsilon_i, \ldots, \epsilon_{n-1}),$$
$$(S_i\sigma)(\epsilon_1, \ldots, \epsilon_{n-1}) = \sigma(\epsilon_1, \ldots, \epsilon_{i-1}, 1, \epsilon_i, \ldots, \epsilon_{n-1}),$$
$$(P_i\sigma)(\epsilon_1, \ldots, \epsilon_{n-1}) = (R_i\sigma)(\epsilon_1, \ldots, \epsilon_{n-1}) + (S_i\sigma)(\epsilon_1, \ldots, \epsilon_{n-1}),$$

where the addition is that of the group Π. The boundary of σ is

$$\partial\sigma = \sum_{i=1}^{n} (-1)^i (P_i\sigma - R_i\sigma - S_i\sigma)$$

where the addition is that of chains in Q; one has $\partial\partial = 0$.

Within the complex Q consider the subcomplex Q_N which is spanned by the "slabs" (all those $n + 1$ cells σ with $n \geq 1$ such that for some index i one has either $R_i\sigma$ or $S_i\sigma$ identically zero) and by the "diagonals" (all those cells of the form $D_i\sigma$ for some i), where

$$(D_i\sigma)(\epsilon_1, \ldots, \epsilon_{n+1}) = \sigma(\epsilon_1, \ldots, \epsilon_i, \epsilon_{i+2}, \ldots, \epsilon_{n+1}), \quad \epsilon_i = \epsilon_{i+1},$$
$$= 0 \qquad \qquad \text{if} \quad \epsilon_i \neq \epsilon_{i+1}.$$

For any abelian group G we then define the *cubical cohomology groups* $Q^n(\Pi; G)$ as the relative cohomology groups of Q modulo Q_N:

$$Q^n(\Pi; G) = H^n(Q, Q_N; G). \tag{8}$$

In particular, equation (6) shows that a 1-dimensional cubical cocycle is a homomorphism, so that $Q^1(\Pi; G)$ is the group of homomorphisms of Π into G. Similarly equation (7) implies that a 2-dimensional cubical cocycle is a (normalized) symmetric factor set $f(x, y)$ of Π in G, so that $Q^2(\Pi; G)$ is the group of abelian group extensions[8] of G by Π. The higher cubical cohomology groups of Π appear to be new.

THEOREM 2. *If $\Pi + \Pi'$ is the direct sum of two abelian groups, there is a natural isomorphism*

$$Q^n(\Pi + \Pi'; G) \cong Q^n(\Pi; G) + Q^n(\Pi'; G).$$

In a subsequent note we shall show that this property, together with the character of the complex Q in the case when Π is an infinite cyclic semigroup, serve to characterize the cubical cohomology groups, in the sense that any other construction for all groups Π of suitable cell complexes with these properties yields exactly the cubical cohomology groups here defined.

THEOREM 3. *There are isomorphisms*

$$H^{m-1+k}[K(\Pi, m); G] \cong Q^k(\Pi; G), \quad k = 1, 2, \ldots, m.$$

The isomorphism θ in question is related to the suspension homomorphism S by $\theta S = \theta$; hence this result at once yields the suspension theorem, for $k \leq m$.

To extend this result to higher values of k, we introduce a sequence of cohomology groups intermediate to the cubical and the ordinary cohomology groups of Π. They are defined by means of subcomplexes Q_t of Q, for $t = 0, 1, 2, \ldots, \infty$. Call an index $i(i = 1, \ldots, n-1)$ *critical* for an $(n + 1)$-cell σ if $R_i S_i \sigma$ is not identically zero; that is, if $\sigma(\epsilon_1, \ldots, \epsilon_{i-1}, 1, 0, \epsilon_{i+2}, \ldots, \epsilon_n) \neq 0$ for some choice of the ϵ's. The *level* of a cell σ is the number of distinct critical indices for σ. The complex $Q_t(\Pi)$ is defined as that subcomplex of $Q(\Pi)$ which is spanned by all cells of level at most t. In particular, if $n \leq t + 1$, any $(n + 1)$-cell σ lies in Q_t. We define the cubical cohomology groups of level t for Π as the relative groups of Q_t modulo $Q_t \cap Q_N$:

$$Q^{n,t}(\Pi; G) = H^n(Q_t, Q_t \cap Q_N; G). \tag{9}$$

The complex Q_∞ is the whole complex Q, hence $Q^{n,\infty}(\Pi) = Q^n(\Pi)$, and also $Q^{n,t}(\Pi) = Q^n(\Pi)$, if $n \leq t + 1$. On the other hand, the $(n + 1)$-

cells of Q_0 have $\sigma(\epsilon_1, \ldots, \epsilon_n) = 0$, unless $\epsilon_1 \leqq \epsilon_2 \leqq \ldots \leqq \epsilon_n$, hence may be mapped in 1-1 fashion on the $(n + 1)$ tuples $[x_1, \ldots, x_{n+1}]$, where $x_i = \sigma(0, 0, \ldots, 0, 1, \ldots, 1)$ with i-1 arguments equal to 1. This correspondence can be used to show that $Q^{n, 0}(\Pi; G)$ is the nth cohomology group of $K(\Pi, 1)$, modulo K_N; hence

$$Q^{n, 0}(\Pi; G) \cong H^n(\Pi; G). \tag{10}$$

Theorem 3 may now be extended as follows.

THEOREM 4. *For all $k = 1, 2, \ldots$ there is an isomorphism*

$$H^{m-1+k}[K(\Pi, m); G] \cong Q^{k, m-1}(\Pi; G).$$

In view of (3), we thus have

THEOREM 5. *If X is an arcwise connected topological space with a given homotopy group π_m and with all other homotopy groups trivial, the singular cohomology groups of X with coefficients in any abelian group G are determined by π_m according to the formulae*

$$H^q(X; G) = 0, \qquad q = 1, 2, \ldots, m - 1,$$
$$H^{m-1+k}(X; G) = Q^{k, m-1}(\pi_m; G), \qquad k = 1, 2, \ldots.$$

The theorem formally includes the case $m = 1$. Briefly, we may say that the ordinary cohomology groups for the *not* necessarily *abelian* fundamental group yield the effect of that group upon cohomology groups of the space, and that the cubical cohomology groups, defined, at suitable levels, for *abelian* groups, provide the corresponding effect for higher homotopy groups.

In a later note we shall give an alternative description of the groups $Q^{k, m-1}(\Pi; G)$ which is more suitable for algebraic computations and topological applications.

* Essential portions of the study here summarized were done during the tenure of a John Simon Guggenheim Memorial Fellowship by one of the authors.

[1] The literature is summarized in Eilenberg, S., *Bull. Am. Math. Soc.*, **55**, 3–37 (1949).

[2] Eilenberg, S., and MacLane, S., *Ann. Math.*, **48**, 51–78 (1947).

[3] Eilenberg, S., and MacLane, S., *Ibid.*, **46**, 480–509 (1945).

[4] Eilenberg, S., and MacLane, S., "Relations between Homology and Homotopy Groups of Spaces, II," *Ibid.*, to appear.

[5] Whitney, H., *Ibid.*, **50**, 285–296 (1949).

[6] Whitehead, G. W., PROC. NATL. ACAD. SCI., **34**, 207–211 (1948).

[7] Freudenthal, H., *Compositio Math.*, **5**, 299–314 (1937).

[8] This is the group Ext (G, π) used in Eilenberg and MacLane, *Ann. Math.*, **43**, 757–831 (1942).

REMARKS ON THE COMPARISON OF AXIOM SYSTEMS

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With the arithmetization of syntax, we can formulate the metalogical problems of consistency, completeness, decidability, relative consistency, etc., as arithmetic problems. On certain occasions this kind of formulation can render the crucial notions more exact. In this note we shall assume that the syntax of each of the systems we consider is arithmetized, and talk in terms of the arithmetic propositions expressing the syntactic properties. In particular, the arithmetic proposition Con(S) expresses the consistency of S. We shall make a few observations on the comparison of systems according to the following three standards (S and S' being two systems): (1) whether Con(S) is provable in S'; (2) whether S is translatable into S'; (3) whether we can obtain in S' a Tarski truth function (or set) for S. We propose first a few definitions.[1]

Definition 1: A system S is said to be translatable into or obtainable within or contained as a part in a system S' if there exists a general re-cursive function T mapping the set of the numbers representing (via the arithmetization) the propositions of S into the set of the numbers representing those of S' such that the set of (the numbers representing) the theorems of S is mapped into the set of those of S' and the image of the negation of a proposition of S is the negation of the image of the proposition.

We note that a function maps the set of the theorems of S into that of S' if and only if axioms of S all correspond to theorems of S' and primitive rules of inference of S all correspond to valid rules of inference of S'. If we assume that systems for various branches of mathematics are given to start with, then the above definition provides us with a more precise meaning to the oft-repeated assertion that mathematics is reducible to or obtainable in logic (that is to say, in certain forms of set theory).

Definition 2: Two systems S and S' are said to be of equal strength if the propositions Con(S) and Con(S') are mutually derivable in number theory. S' is said to be stronger than S if Con(S) is derivable from Con(S') in number theory but not conversely.

Definition 3: A system which contains number theory as a part is said to be a mathematical system. (In particular, a system of number theory is a mathematical system.) If Con(S) is a theorem of number theory, then S is said to be an elementary system.

Using these definitions, we can prove the following theorems:

THEOREM 1. *If S is translatable into S', then Con(S) is derivable from Con(S') in number theory. If S and S' are translatable into each other, then*

they are of equal strength. If S is stronger than S', then S is not translatable into S'.

Proof: The translation of a proof of S that gives a contradiction is a proof of S' which also gives a contradiction, and vice versa.

THEOREM 2. *All consistent, decidable systems are elementary, and therefore of equal strength.*

Proof: Following a by now established usage, we call a system S decidable if the class of its theorems is general recursive. Let S be a system which is both consistent and decidable. Since all general recursive functions are definable in number theory, there exists a function f for which we can prove in number theory either that $f(n)$ is 0 or that $f(n)$ is 1 according as n represents a theorem of S or not. But S is consistent. Hence, there is a number k which represents a proposition of S which is not a theorem. Therefore, we can prove in number theory that $f(k)$ is 1. Hence, the proposition $\text{Con}(S)$ is a theorem of number theory.

Thus, from results of Post, Löwenheim, Presburger and Skolem, we know that propositional calculus, monadic restricted functional calculus, arithmetic with just addition and arithmetic with just multiplication are all elementary systems. However, not all elementary systems are decidable. One case is the restricted functional calculus which has been shown by Church to be undecidable. The usual proof for its consistency[2] amounts to showing that it is translatable into the propositional calculus. Hence, by Theorem 1, it is an elementary system, and we have:

THEOREM 3. *There exist elementary systems which are not decidable.*

According to a theorem of Gödel, if S is a mathematical system, then $\text{Con}(S)$ is not a theorem of S (unless S is inconsistent). Hence, if $\text{Con}(S)$ is a theorem of a mathematical system S', then $\text{Con}(S')$ is not derivable from $\text{Con}(S)$ in S' or in number theory (unless S' is inconsistent). On the other hand, if S' contains S as a part, by Theorem 1, $\text{Con}(S)$ is derivable from $\text{Con}(S')$ in number theory. Hence, we have:

THEOREM 4. *If S' is consistent and contains a mathematical system S as a part and Con(S) is a theorem of S', then S' is stronger than S.*

By extending somewhat a formulation[3] of the well-known theorem on denumerable models (Löwenheim-Skolem-Herbrand-Gödel), we can prove the following theorem:

THEOREM 5. *Every system S is translatable into the system obtained from number theory by adding Con(S) as a new axiom.*

Let L be a system of set theory with finitely many special axioms such that number theory as well as a theory of the classes of natural numbers can be obtained in it.[4] Suppose x, y, z, etc., are the variables of L (or the variables of the highest type in L). Let L'' be a system related to L as a functional calculus of order n is related to one of order $n - 1$, with the new variables X, Y, etc., and L' be the system which is like L'' except that the

new variables are not allowed in the definitions of classes of lower types. Thus, both L' and L'' contain a principle stating that for every propositional function (it may contain bound variables of the type just introduced) ϕx, there exists a class Y such that x belongs to Y if and only if ϕx. But L'' contains in addition also principles such as: for every propositional function of L'', there exists a class c (of a type in L) defined by it. Let further $L\#$ be the system obtained from L by adding $\text{Con}(L)$ as a new axiom. We want now to consider the interrelationships among the systems L, L', L'' and $L\#$.

We observe first that with the methods developed by Tarski and others, we can give a truth set for L in L'' and prove $\text{Con}(L)$ in L''. Moreover, since $\text{Con}(L)$ is a theorem of L'', we can prove in L'' that the number representing the proposition $\text{Con}(L)$ also belongs to the truth set so that $\text{Con}(L\#)$ is also a theorem of L''.

THEOREM 6. *Both $\text{Con}(L)$ and $\text{Con}(L\#)$ are theorems of L''. Hence, L'' is stronger than $L\#$.*

By applying Theorem 5, we can prove that the mathematical system L' is translatable into $L\#$. Hence, we have:

THEOREM 7. *The proposition $\text{Con}(L')$ is derivable from $\text{Con}(L\#)$ in number theory.*

It follows that $\text{Con}(L\#)$ is not provable in L' unless L' is inconsistent. Let us examine now why we cannot prove $\text{Con}(L\#)$ in L'.

It is not hard to obtain within L' a Tarski truth set for both L and $L\#$. Thus, let P_m be the propositional function represented by the number m. We want to prove:

THEOREM 8. *There exists a class Tr in L' such that for every given proposition P_m of L and $L\#$, we can prove in L' a theorem of the form (S): $m \, \epsilon \, Tr$ if and only if P_m.*

The proof may be outlined as follows. For brevity, let us assume that L contains only one kind of variable x, y, etc., which may be written alternatively as v_1, v_2, etc. Following Professor W. V. Quine, we shall identify the infinite sequences of classes of L with the classes x of L such that x is a class of ordered pairs whose second terms are all positive integers. Let us use the letter g as a variable ranging over such classes of L. Then we can define the jth term g_j of an infinite sequence g, and the infinite sequence $t(g, n, y)$ obtained from g by substituting the class y for g_n. With these notions we can define a propositional function $R(k, X)$ of L' as follows: For all g and m, a pair $g;m$ belongs to X if and only if P_m is a propositional function of L containing no more than k logical operators and is such that either (1) P_m is $v_n \, \epsilon \, v_j$ and $g_n \, \epsilon \, g_j$, or (2) P_m is $P_n \downarrow P_j$ (neither P_n nor P_j) and $g;n \, \epsilon \, X \downarrow g;j \, \epsilon \, X$, or (3) P_m is $(v_n)P_j$ and $(y)(t(g, n, y));j \, \epsilon \, X)$. Then Tr is just the class of natural numbers m such that (g) $(EX)(g;m \, \epsilon \, X$ and $R(m, X))$. But for each given number m, we can

prove.in L' the propositions: (1) $(EX)R(m, X)$; (2) for all Y and Z, if $R(m, Y)$ and $R(m, Z)$, then $Y = Z$. Therefore, we can prove in L' all the special cases falling under the schema (S).[5]

If we can prove in L' also that all theorems of L belong to Tr, then we can easily prove Con(L) in L'. Let us assume that the proofs of the theorems of L are enumerated in some way so that each proof is either (1) an axiom of one of the finitely many kinds of axioms of the restricted functional calculus, or (2) one of the finitely many special axioms of L, or (3) the result obtained from one or two previous proofs by adding one line in accordance with the ordinary rule of generalization or that of modus ponens. Let $b(n, m)$ be the proposition of L' stating that the nth proof in the above enumeration is a proof of P_m, and k be the number of the proposition $0 \neq 0$ of L. Then we can prove easily in L' the propositions (1) k does not belong to Tr, and (2) if $b(1, k)$ then k belongs to Tr. Moreover, since L contains only a finite number of special axioms, we can also prove in L' the following proposition: $(i)((n)((n \leq i$ and $b(n, k)) \supset k \epsilon Tr) \supset (b(i + 1, k) \supset k \epsilon Tr))$. By combining this with the last two theorems of L', we can prove in L': $\sim b(1, k)$ and $(i)((n)(n \leq i \supset \sim b(n, k)) \supset \sim b(i + 1, k))$. However, it does not follow that we can also prove in L' the proposition $(m) \sim b(m, k)$ which is equivalent to the proposition Con(L). In order to make the inference, we have to apply the induction principle saying that every class X (in L') of natural numbers has a least member. Since L contains number theory, we know that every class x (in L) has a least member. But we cannot infer that every class X (in L') of natural numbers also has a least number, unless we should assume (as in L'') that large variables are allowed in defining classes x, y, etc., and therefore every class X of natural numbers is identical with a class x of natural numbers.

Indeed,[6] if we could make the inference and prove Con(L) in L', Con$(L\#)$ would also be a theorem of L' and L' would be inconsistent by Theorem 7. Thus, by the schema in Theorem 8, if Con(L) were a theorem of L', then we could prove in L' that the number representing Con(L) belongs to Tr, and therefore that all the numbers representing theorems of $L\#$ belong to Tr. Then it would follow immediately that Con$(L\#)$ is a theorem of L'.

Since we assume in L number theory and a theory of classes of natural numbers, the number 0, the success or function $+1$, and the class Nn of natural numbers are all defined in L. In the arguments of the last two paragraphs we are assuming that these same definitions of L are also employed in L'. Let us now retain in L' the definitions of 0 and $+1$ used in L but redefine the class Nn as the intersection of all classes X such that 0 belongs to X and for every n, $n + 1$ belongs to X if n does. Then we can derive in L' the principle of induction for all classes of L' from the principle of class formation. Hence, we can prove in L' the arithmetic

•

proposition expressing the consistency of L. However, since the definition of the class of natural numbers in L' is different from that in L and $L\#$, the proposition we prove as a theorem in L' is different from the axiom of $L\#$ which also expresses the consistency of L. Therefore, although we can prove in L' a proposition $Con(L)$ expressing the consistency of L, we cannot use the argument mentioned above to derive $Con(L\#)$ in L'.

Summing up the last few paragraphs, we have:[7]

THEOREM 9. *If L' is consistent and we use in L' the same definitions of $0, +1$, and Nn as in L and $L\#$, then (1) the principle of induction is independent of the axioms of L' and, in particular, there exists a propositional function $\phi(i)$ of L (viz., the propositional function $(n)(n \leq i \supset \sim b(n, k)))$ such that $\phi(0)$ and $(i)(\phi(i) \supset \phi(i + 1))$ are provable in L' but $(m)\phi(m)$ is not; (2) although there is a truth set for L in L', $Con(L)$ is not provable in L'.*

THEOREM 10. *By choosing suitable definitions for natural numbers, we can prove in L the proposition $Con(L)$. But $Con(L\#)$ is not a theorem of L' unless L' is inconsistent.*

At several places Skolem stresses that the meaning of mathematical concepts (such as the concept of "natural number") is relative to the meaning of the concept of "set." The above two theorems seem to provide a rather remarkable illustration of Skolem's point.

We proceed to state a number of corollaries of the Theorems 1–10.

THEOREM 11. *$Con(L')$ is a theorem of L''.*

Proof: By Theorem 6 and Theorem 7.

THEOREM 12. *L'' is stronger than L' and therefore not translatable into L'.*

Proof: By Theorem 11, Theorem 4 and Theorem 1.

THEOREM 13. *L' is stronger than L and therefore not translatable into L.*

Proof: By Theorem 10, Theorem 4 and Theorem 1.

THEOREM 14. *Although there is a truth set for $L\#$ in L', L' is translatable into $L\#$.*

Proof: By Theorem 8 and the proof of Theorem 7.

There seems to be a wide-spread impression that whenever we can obtain in a mathematical system S' a truth set for a system S, we can also prove $Con(S)$ in S'. Since the proof of $Con(S)$ depends more heavily on what axioms S contains than the definition of a truth set for S, there is not much reason to suppose that this should be true in general. And our results above provide us with a counter-example. Thus, by Theorem 8 and Theorem 10, we have:

THEOREM 15. *If L' is consistent, then $Con(L\#)$ is not provable in L' although there is in L' a truth set for $L\#$.*

On the other hand, since a truth definition for L is also a truth definition for $L\#$, by Tarski's theorem stating that we cannot define truth of a mathematical system within the system itself, we have:

THEOREM 16. *Although Con(L) is a theorem of L⧸, we cannot obtain in L⧸ a truth definition for L.*

It is a pleasure to acknowledge here our indebtedness to Professors P. Bernays, W. V. Quine and J. B. Rosser for valuable suggestions and criticisms.

[1] Questions of equivalence and translatability among systems have been studied by numerous authors including R. Carnap (*Logical Syntax of Language*, London, 1937) and John G. Kemeny (*J. of Symbolic Logic*, 13, 16–30 (1948); *Thesis*, Princeton University, 1949). The definitions we adopt in this note seem to differ from theirs considerably, probably because they are interested in more general problems. Many problems on the truth set *Tr* of a system have been treated carefully by A. Tarski in *Studia Philosophica*, 1, 261–405 (1936).

Throughout this note, we shall mean by number theory a system which contains, beyond the restricted functional calculus and the theory of identity for natural numbers, the Peano axioms and the recursive equations for addition and multiplication.

The Definition 1 is quite liberal and for certain purposes, it may be desirable to add (as we have done at another place) a condition on the function T stating that the image of the conjunction of two propositions of S is the conjunction of their images. It will then follow that similar things hold for all the truth functions. Such a change in Definition 1 would not affect the proof of any of the theorems in this note.

Following a prevalent usage, we assume that the notions of proposition, axiom and immediate consequence in every *system* are all general recursive. In what follows, the translation of a proof will be understood as consisting of the translations of all the lines of the proof. It is not hard to see that if S is translatable into S' according to Definition 1, then there is a general recursive function mapping proofs in S onto their translations in S'.

[2] Hilbert and Ackermann, *Grundzüge der theoretischen Logik*, Berlin, 1938, p. 70.

[3] Hilbert and Bernays, *Grundlagen der Mathematik*, vol. 2, 1939, pp. 234–250. Details of proofs for theorems similar to the theorems 1, 5, 7, 8 and 10 of the present note are given in a longer paper of mine (entitled "On the Consistency Question of Analysis") which will probably be published in the future.

[4] For example, the system in Gödel, K., *The Consistency of the Continuum Hypothesis*, Princeton, 1940, or the alternative formulation given by the present author in these PROCEEDINGS, 35, 150–155 (1949). We may take as L either the system determined by N1-N7 in that note or any subsystem of it containing roughly axioms answering to I–III, Va and VI in Bernays, P., *J. of Symbolic Logic*, 2, 65–77 (1937); 6, 1–17 (1941).

[5] We want to thank Professor P. Bernays for suggesting to us the present form of the set *Tr* which is more convenient than the definition of *Tr* we used before. It may be noted in passing that if instead of the class *Tr* we take the proposition function (*g*) (*EX*) (*g;m ε X* and *R(m, X)*) as *Tr(m)*, we can also prove the following theorem: In the system obtained from L' by omitting all the classes X not definable without using bound large variables, we can prove, for each given proposition P_m of L and $L⧸$, a theorem stating that *Tr(m)* if and only if P_m. In other words, if we want, instead of a true set, merely a truth definition for L and $L⧸$, it suffices to use a system weaker than L'.

[6] The argument of this paragraph was communicated to us by Dr. John G. Kemeny in conversation.

[7] The following two theorems are due essentially to Professor J. Barkley Rosser who makes these points clear to us in criticizing our earlier attempts to prove the inconsistency of L' by the methods sketched in this note.

POTENTIAL CHANGES IN CHLOROPLAST SUSPENSIONS AND IN WHOLE CYTOPLASM PREPARATIONS OF PLANT CELLS ON ILLUMINATION* †

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The investigation of the intermediate steps in the photosynthetic process has been difficult because of the inability to obtain photosynthesis in cell-free preparations. Any treatment which ruptures the plant cell membrane results in an almost complete loss of photosynthetic activity.[6] However, it appears that part of the photosynthetic reaction can be obtained with isolated chloroplasts or chloroplast fragments in the presence of added oxidants.[3] Upon illumination of such a system, water is oxidized with the production of oxygen gas and hydrogen ion while the added oxidant is reduced. No carbon dioxide is fixed in such preparations.[2] This reaction is now generally termed the Hill Reaction[6] and may be represented by the following equation when ferric iron is the added oxidant:

$$4Fe^{+++} + 2HOH \rightarrow 4Fe^{++} + O_2 \uparrow + 4H^+. \qquad (1)$$
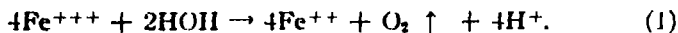
Effective oxidants appear to include ferric oxalate,[3] p-benzoquinone,[10] various substituted quinones,[1] ferricyanide, chromate, and certain redox indicators.[5]

In past work the rate of the Hill reaction has been measured by observing oxygen evolution,[6] the reduction of methemoglobin,[3] hydrogen ion formation[4] and decolorization of redox indicators.[7] It appears from equation 1 that a change in the oxidation-reduction potential should occur as the reaction proceeds, which could be measured potentiometrically by the introduction of suitable electrodes into the chloroplast preparation. Since oxidation-reduction potentials can be determined rapidly and accurately this would appear to be a useful method for observing the reaction. In

the literature available to the authors, no reports of such a technique were found as applied to cell-free preparations, although potential changes upon illumination of living purple (photosynthetic) sulfur bacteria[5] and the algae Chlorella[6] have been observed.

Materials and Methods.—Whole cytoplasm preparations were made by blending green leaves in a small amount of isotonic sucrose in a Waring blendor. The blendate was filtered and briefly centrifuged to remove

TIME (MINUTES)
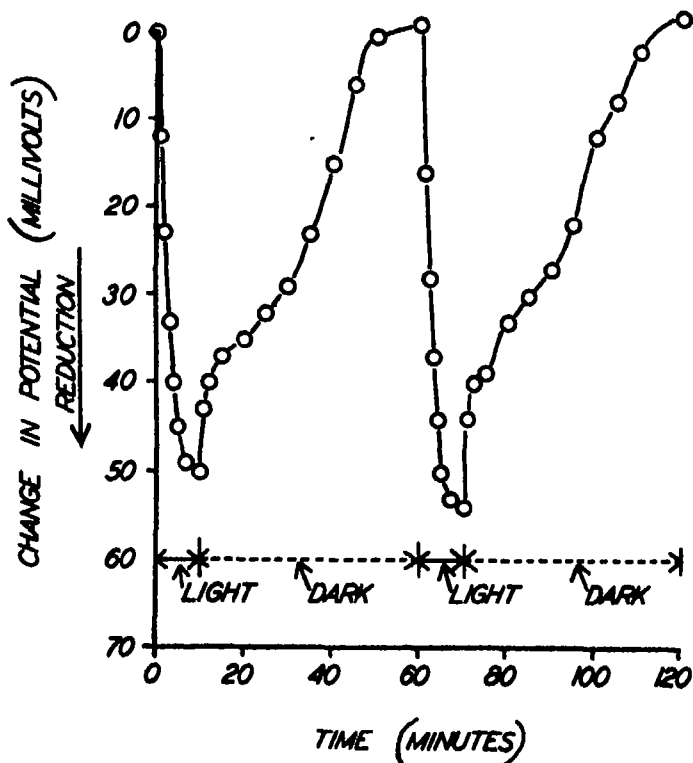
FIGURE 1

Curve showing potential changes in light and in dark of whole cytoplasm preparations of leaves of the wild sunflower (*Helianthus annuus*, L.) without added oxidant.

intact cells and large fragments of tissue. Chloroplasts were isolated by blending leaves as described above and then fractionating the blendate by differential centrifugation. The chloroplasts were resuspended in isotonic sucrose for use. For measurements the preparations were placed in small lucite cells provided with a back of platinum foil which served as one of the electrodes. A small hole connected the chamber of the cell to a side arm in which was inserted a small Beckman calomel electrode.

Experiments were routinely carried out at 4°C., and proper precautions were taken to prevent temperature changes in the cell during the illumination period.   In most of the experiments the light was filtered through
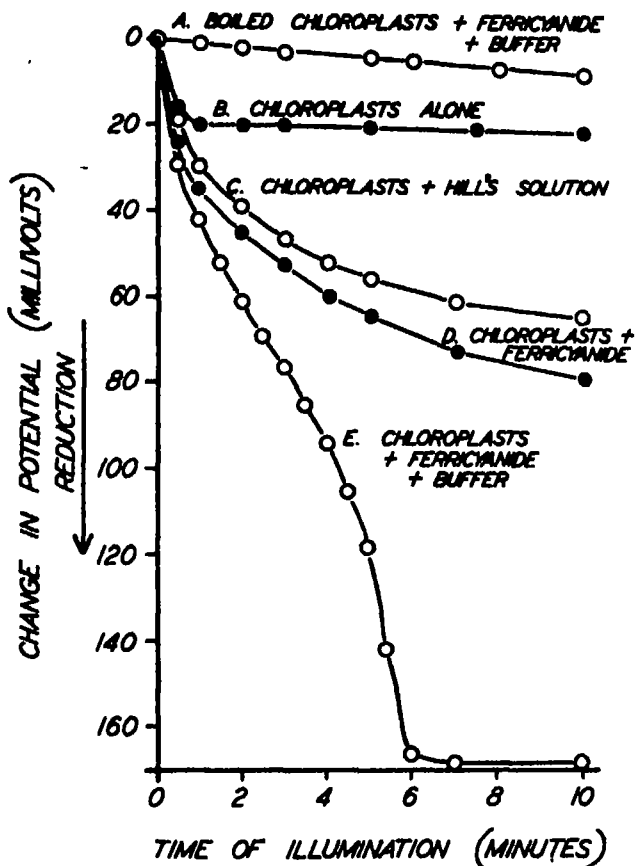
FIGURE 2

Curves showing the changes in potential upon illumination of suspensions of washed spinach chloroplasts under different conditions as indicated.   Chloroplast concentration equal to 150 mg. of chlorophyll per liter.   Potassium ferricyanide concentration 0.001 *M* where used.   Potassium phosphate buffer concentration 0.1 *M*, pH 6.7, where used.   Hill's solution as described in reference 4.

a Wratten A (red) filter which cut off all wave-lengths shorter than 5800 Å. Intensity of red light on the cell surface was approximately 5000 lux.

Whole Cytoplasm of Sunflower Leaves.—These preparations exhibited potential changes upon illumination as shown in figure 1.   As the curve

indicates, there is a rapid change in potential upon illumination. The direction of the change shows that reduction is occurring. When the light is turned off, there is a more gradual change of the potential in the opposite direction, which levels off at a value near the original potential of the preparation in the dark. If the light is turned on again, the potential rapidly changes as before, and when the light is turned off the potential again returns to its original dark value. This cyclic process can be repeated for a number of hours at a temperature of 25°C. before the preparation loses its activity. Activity is immediately destroyed by boiling. This system is of interest because it appears to contain a naturally occurring oxidant. In addition, the system is reversible, which is what might be expected if such a system were operating in photosynthesis to carry energy from light absorbing regions of the cell to regions where reduction of carbon dioxide could occur. None of the other species of plants investigated to date show this activity in the absence of added oxidant.

Results with Isolated Spinach Chloroplasts.—Isolated chloroplasts from spinach leaves obtained on the local market were prepared as described above. Chloroplasts suspended in whole cytoplasm showed no activity, and chloroplasts suspended in isotonic sucrose exhibited only a slight, essentially irreversible, activity as shown in curve B of figure 2. Chloroplasts suspended in Hill's solution[4] showed a greater potential change on illumination as indicated in curve C of figure 2. Red light was used for these determinations as Hill's solution was rapidly reduced by white light of the intensities employed. Hill's solution is a fairly complicated mixture and the interactions of the various components during photochemical reduction are not well established. Thus it became desirable to use a medium containing a single reducible component so that calculations could be made concerning concentrations of materials from the observed potentials.

Several workers have reported ferricyanide alone to be a satisfactory oxidant in place of Hill's solution. This was also found to be true in the present work. Curve D in figure 2 shows the potential change upon illumination of spinach chloroplasts suspended in 0.001 M potassium ferricyanide. Red light was used in all experiments with ferricyanide as an oxidant, as ferricyanide alone showed a potential change when illuminated with white light. Curve A, figure 2, shows that boiling the chloroplasts destroyed all of the activity of this system.

A consideration of equation 1 shows that hydrogen ion is produced as the reaction proceeds. In unbuffered preparations this results in a rapid decrease in pH which inactivates the system. Curve E of figure 2 shows the potential change in a system containing spinach chloroplasts and 0.001 M ferricyanide buffered at pH 6.7 with 0.1 M phosphate buffer. The potential-time curve given by unbuffered systems is a typical logarith-

mic curve, while the buffered system gives a typical potentiometric titration curve plus a region where the potential rapidly assumes a constant value.
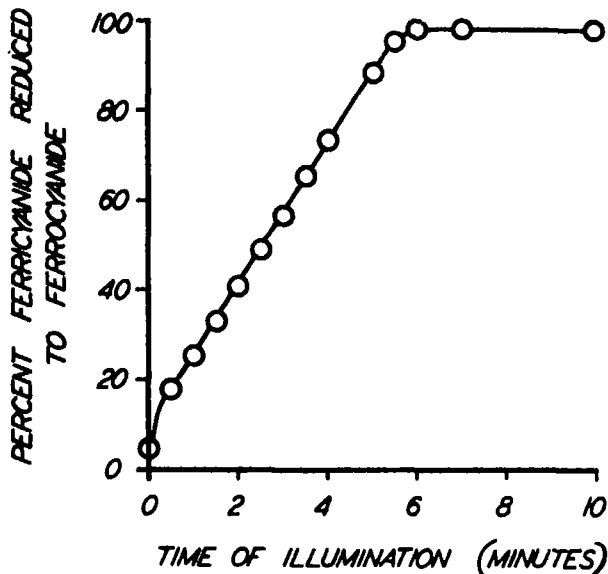
FIGURE 3

Curve showing per cent reduction of ferricyanide to ferrocyanide with time by washed spinach chloroplasts upon illumination. This curve is derived from the data of curve E of figure 2 by applying equation 4.

The amount of the ferricyanide originally present which is reduced to ferrocyanide with time may be calculated from the potential-time data as follows:

$$\text{ferrocyanide} \rightarrow \text{ferricyanide} + e \tag{2}$$

$$E = E^o - \frac{RT}{F} \ln \frac{(\text{ferricyanide})}{(\text{ferrocyanide})} \tag{3}$$

$$\% \text{ ferricyanide reduced to ferrocyanide} = 100 \times \frac{1}{e^{-(F/RT)(E-E^o)} + 1} \tag{4}$$

where E is the observed potential, E^o is the standard half-cell potential for the ferricyanide-ferrocyanide couple, F is the Faraday of electricity, R is the gas constant and T is the absolute temperature. Equation 4 may then be used to calculate the per cent reduction of ferricyanide correspond-

ing to each observed potential. The potential-time data of curve *E* of figure 2 are shown, as calculated on this basis, in figure 3. The conversion of ferricyanide to ferrocyanide is linear with time upon illumination up to a conversion of approximately 97% when the curve rapidly flattens. The slope of this curve may be used as a measure of the rate of the reaction. Rates determined by this technique are highly reproducible, the maximum variability of duplicate runs being 2%. The method has been used to determine the effects of chloroplast concentration, ferricyanide concentration, temperature, pH, buffer concentration, cytoplasm concentration, inhibitors, methods of preparing chloroplasts, electrode area, electrode position and light intensity on the rate of reduction of ferricyanide by chloroplast suspensions on illumination. This work will be reported in detail elsewhere.

The rapidity, high accuracy and reproducibility of the method coupled with the fact that it depends on concentration ratios of oxidized and reduced forms of compounds rather than on absolute concentrations (which permits an extremely high sensitivity), suggests its use in such matters as the determination of quantum efficiencies, the determination of the number of photons required per unit process per photoreduction center, and the determination of the kinetic interrelation between the light absorption process and the oxidation of water under both steady state and non-steady state conditions.

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⁴ Holt, A. S., and French, C. S., *Arch. Biochem.*, 9, 25 (1946).

⁵ Holt, A. S., and French, C. S., *Ibid.*, 19, 368 (1948).

⁶ Holt, A. S., and French, C. S., *Photosynthesis in Plants*, Ch. 14, Iowa State College Press, 1949.

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¹⁰ Warburg, O., and Lüttgens, W., *Naturwiss.*, 32, 161 (1944).

THE STRUCTURE OF THE $Mo_6Cl_8^{--}$ COMPLEX IN THE CRYSTAL $(NH_4)_2(Mo_6Cl_8)Cl_6 \cdot H_2O$

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Cyrill Brosset has determined the crystal structures of the bipositive molybdenum compounds $(Mo_6Cl_8)(OH)_4 \cdot 14H_2O^1$ and $(Mo_6Cl_8)(Cl_4 \cdot 2H_2O) \cdot 6H_2O^2$ and has found that the basic structural unit for these compounds is a $(Mo_6Cl_8)X_4$ group in which X is oxygen or chlorine. The structure of this group can be visualized as follows (Fig. 1). The molybdenum atoms are at the corners of a nearly regular octahedron of edge 2.63 Å, eight chlorine atoms are at the corners of a symmetrically circumscribed cube such that the shortest Mo–Cl distance is 2.56 Å, and the six X atoms are at the corners of a larger octahedron, one X being bonded to each molybdenum atom.

Some years ago, at the suggestion of Professor Linus Pauling, I began an x-ray study of the compound $(NH_4)_2(Mo_6Cl_8)Cl_6 \cdot H_2O$. It forms crystals with space group C_s^4-Cc and unit cell parameters $a_1 = 19.33$ Å, $a_2 = 14.93$ Å, $a_3 = 9.16$ Å, and $\beta = 115.2°$. The volume of the unit cell is 2390 Å,³ which, for four formulae per cell, leads to calculated density 3.12 g. cm.$^{-3}$, in good agreement with the observed density 3.09 g. cm.$^{-3}$, determined by flotation.

By applying the radial distribution method to a powder photograph of this compound, I have found that it contains the group $(Mo_6Cl_8)Cl_6^{--}$ with the structure described above. The intensity function used was

$$I'(s) = \sum_i I_i e^{-C(s-s_i)^2}, \tag{1}$$

where I_i is visually estimated intensity of the ith powder line and $s = \dfrac{4\pi \sin \theta}{\lambda}$, θ being the Bragg angle. It is readily shown that, to a good approximation, $I'(s)$ is given by

$$I'(s) = K \sum_{i,j} A_{ij} \sin sr_{ij}, \tag{2}$$

where

$$A_{ij} = \frac{\overline{f_i f_j}}{r_{ij}} e^{-(r_{ij}^2/4C)}.$$

The radial distribution integral was then calculated from the equation

$$rD(r) = \int_0^{s_{max.}} e^{-as^2} I'(s) \sin sr \, ds \tag{3}$$

by means of the punched card technique developed in these Laboratories for electron diffraction investigations.[3] The radial distribution function is then interpreted in terms of the structure by the relationship

$$rD(r) = \sum_{i,j} \frac{A_{ij}}{4} \sqrt{\frac{\pi}{a}} e^{-[(r-r_{ij})^2/4a]}, \tag{4}$$

the sum being over all of the non-zero distances in the proposed structure and A_{ij} being defined above. The function $I'(s)$ has the advantage that it is a smooth function of s and can therefore be treated in much the same
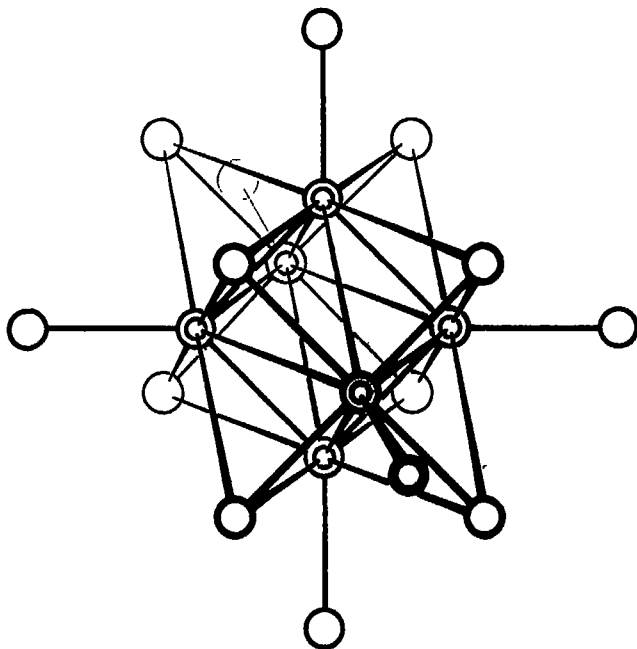
FIGURE 1

The $(Mo_6Cl_8)Cl_6^{--}$ ion, double circles are Mo atoms; single circles are chlorine atoms.

way as an electron diffraction scattering curve; for instance, theoretical $I'(s)$ curves can readily be calculated for any proposed structure by means of equation (2). The function $I'(s)$ and the radial distribution function obtained therefrom are shown in figure 2. The $(Mo_6Cl_8)Cl_6^{--}$ group would probably have, following the work of Brosset, the following parameters:

Shortest Mo–Mo	= 2.63 Å (average)
Mo–Cl (cubic Cl)	= 2.56 Å (average)
Mo–Cl (octahedral Cl)	= 2.43 Å

A radial distribution function corresponding to this model calculated from equation (4) is shown in figure 2. The agreement with the experimental radial distribution function is good, the only serious discrepancy out to about 5 Å being the greater height of the peak near 3.6 Å in the experimental curve. This is explained by the fact that there are additional
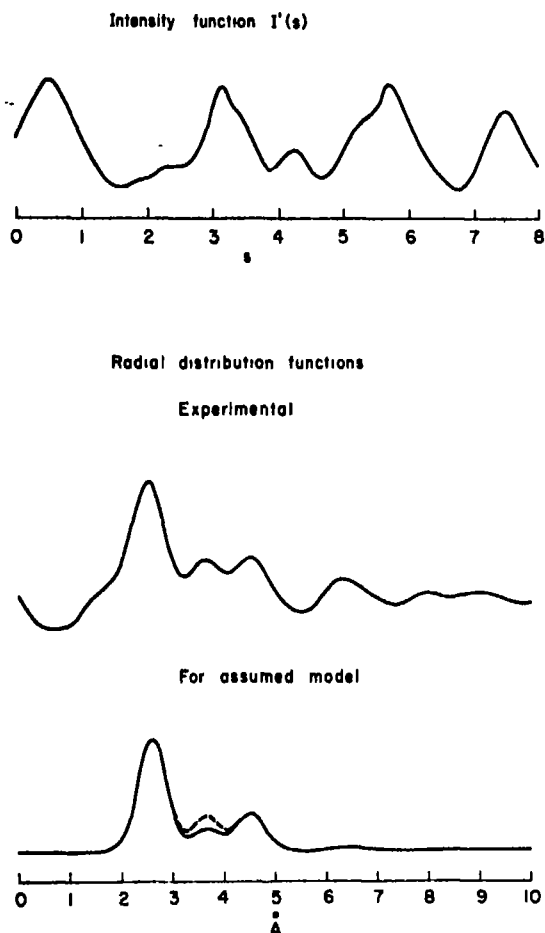
FIGURE 2

Intensity and radial distribution functions for
$(NH_4)_2(Mo_6Cl_8)Cl_4 \cdot H_2O$.

Cl...Cl distances of about 3.60 Å in the crystal due to intermolecular contacts. If the reasonable assumption is made that each octahedral chlorine atom makes van der Waals' contact with six chlorines in different molecules and that each cubic chlorine atom makes contact with three,

the theoretical radial distribution function becomes as indicated by the dotted line in figure 2; the heights are now in excellent agreement. The positions and relative heights of the maxima less than 5 Å in the two radial distribution functions are

EXPERIMENTAL		THEORETICAL	
HEIGHT	POSITION	HEIGHT	POSITION
100	2.53 Å	100	2.59 Å
31.8	3.63 Å	32.0	3.65 Å
33.6	4.50 Å	34.4	4.50 Å

The agreement provides very strong evidence that the $(Mo_6Cl_8)X_6$ group found by Brosset is present in $(NH_4)_2(Mo_6Cl_8)Cl_6\cdot H_2O$.

Space group and packing considerations lead to the conclusion that the $(Mo_6Cl_8)Cl_6^{--}$ groups are arranged in nearly directly superposed hexagonal layers parallel to (001). Each group has four neighboring groups at about 8.75 Å and two at 9.16 Å in the same layer; the interlayer spacing is 8.76 Å.

* Contribution No. 1432.
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FACTORS GOVERNING SEXUAL SELECTION AS AN ISOLATING MECHANISM IN THE POECILIID FISH LEBISTES RETICULATUS

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In a previous paper[1] it was pointed out that three species of small viviparous Poeciliid fishes, *Poecilia vivipara*, *Micropoecilia parae*, and *Lebistes reticulatus*, form an unusually intimate sympatric association in certain brackish coastal waters of the island of Trinidad. The geographic range of all three species is similar, *P. vivipara* and *L. reticulatus* being characteristic inhabitants of the coastal streams and lagoons of northeastern South America and the immediately adjacent islands, while *M. parae* is represented in the same mainland areas either by the typical species or by morphologically distinguishable but closely related forms. The ecological preferences of the three species are closely similar. All are primarily surface feeders in warm shallow waters. In Trinidad, *P. vivipara* and *M. parae* are confined to brackish coastal waters, while *L. reticulatus* is a common inhabitant of all bodies of water of any size, ranging from mountain streams

and inland ponds almost into the open sea. In the areas where their ranges coincide, the three species swim in mixed schools and feed in close association.

The females of all three species are generally similar in form, coloration, and habitus. All three species show pronounced sexual dimorphism, with brilliantly colored males which, unlike the females, differ markedly between species, both in form and coloration and in the details of courting and mating patterns.

Reproductive isolation between the three species appears to be essentially complete in nature. No intergrades have been found in any of the populations examined, amounting in total to approximately three thousand individuals. Hybrids between *P. vivipara* and *L. reticulatus* have been obtained in the laboratory, but only with difficulty. Though large and vigorous, they were apparently sterile. It is clear, therefore, that physiological isolating mechanisms are of importance in maintaining the species structure of the population.

The pronounced sexual dimorphism of the three species and the elaborate color and courtship patterns of the males suggest that sexual selection may also be of importance as an isolating mechanism in this sympatric association. Experiments reported in the paper referred to above indicated that the male of *L. reticulatus* may indeed exhibit a remarkably high degree of selectivity for conspecific females in mixed populations which have been in equilibrium for some time. No evidence was found in any of these observations, however, that the females of any of the three species exhibit a similar selective response to the appropriate males. Thus the predominant, if not the whole, share of sexual selection appears initially to reside with the male.

Two points of considerable interest emerge from this picture, neither of which could be satisfactorily settled from the data earlier presented. First, it seemed *a priori* somewhat unlikely that the high order of specificity in mate-selection which was found should rest with the male alone. It would seem more probable that the female exhibits complementary selective reactions, the whole behavior pattern of the two sexes then forming a chain of stimulation, such as has been reported by Tinberger and Van Iersel[3] for the stickleback. Certainly such complementary reactions in the female Lebistes are by no means evident, but it is entirely conceivable that they are of a kind not easily detected by the observer. The matter is of considerable importance in the interpretation of the evolutionary significance of the elaborate male patterns of color and behavior in these forms.

Second, it was observed in the earlier work that the choice-pattern of the male Lebistes was characteristically quite inefficient at the beginning of an experiment, and increased in accuracy rapidly as the experimental situation stabilized, reaching to as high as 95.5% of correct choices after one week.

This situation is susceptible of two quite different interpretations. On the one hand, it may be interpreted as the simple expression of an innate instinctive preferential reaction of male Lebistes to females of their own species which became progressively more clearly expressed as the males became adjusted to the environment of the experiments, this behavior replacing a higher, more generalized excitability in which random contacts tended to occur. Such an instinct pattern, of course, might be expected to be positively selected in evolution if interspecific sterility was general, or if the interspecific FI hybrids showed lowered fertility over either parent in accord with the general principle of "conservation of gametes" originally stated by Dobzhansky and recently further elucidated by Mayr.[3] Both these conditions are fulfilled in populations of *P. vivipara, M. parae,* and *L. reticulatus.*

On the other hand, the improvement in efficiency of the male pattern of choice with time may result from a learning process, responsive to progressive conditioning of the male to "correct" as opposed to "incorrect" gonopodial contacts.

Both of these points are of considerable theoretical interest. Neither can be directly tested by experiments of the kind earlier described because of the factor of interspecific sterility. It seemed possible, however, that light might be shed on both questions through experiments of similar design in which the females of *M. parae* and *P. vivipara* were replaced by mutant females of Lebistes differing from the normal wild type only in single genes affecting body coloration. Races of Lebistes are available which exhibit such differences but are entirely interfertile, both among themselves and with the wild type, and in which the FI progeny exhibit fertility and viability differing little if any from that of the parents. The males of such races, moreover, exhibit courting and mating patterns identical with the wild type, and in permanently mixed cultures both sexes breed as readily with the wild type as within their own race. Work of this sort has proved feasible and suggestive.

The Experimental Material.—Nearly all the known genes for body coloration in Lebistes are both sex-linked and sex-limited, being expressed only in the adult male. A few, however, affecting the general body-color background rather than the male color patterns, are autosomal and are fully expressed in adults and young of both sexes. Two of these, named by Goodrich "Golden" and "Cream," were selected for this work.[4] Golden behaves as a single-factor autosomal Mendelian recessive to wild type, while Cream is allelic and recessive to it and to wild type. The mutation Golden in the homozygous condition produces a fish of bronze but not translucent ground-color, showing scattered patches of melanic pigment in the body and fins. Melanin is accumulated especially about the edges of the scales and in the underlying diamond pattern, resulting in a pro-

nounced accentuation of the normal reticulate markings of Lebistes. The mutation Cream when homozygous leads to an almost complete suppression of melanin in skin pigmentation. Frequently no pigmented melanophores are macroscopically visible, although a few are probably always present. As a result, Cream fish are without reticulation and of a translucent light yellow coloration. The males of both races exhibit typical sex-linked color patterns superposed on these backgrounds, in so far as the limited melanin available will permit. Both races are of normal form and behavior, differing from the species only in the rather marked alterations of background body color, which contrast conspicuously with the gray reticulate pattern of the wild type. Heterozygotes of both races with wild type are phenotypically indistinguishable from homozygous wild type. Heterozygotes of Golden and Cream are indistinguishable from Golden. Females of wild type, Golden, and Cream stocks carrying known sex-linked factors for male coloration (which of course were not phenotypically expressed) were used throughout the work.

Males of several stocks were used. Wild males or their progeny were drawn from stocks taken from the Maracas and Arima Rivers, the Nariva Swamp, and at Maracas Beach in Trinidad, and from water courses near Delaford in Tobago. The first two locations in Trinidad and the one in Tobago were freshwater streams where Lebistes normally occurs in the absence of the other species, while the latter two locations in Trinidad were brackish lagoons in which all three forms occur sympatrically.

In addition to these individuals from wild cultures taken directly from Trinidad and Tobago, males from other stocks were used. These included wild-type males from an aquarium race carrying the sex-linked gene Pauper, first obtained by Winge in 1922 from the West Indies, described by him in 1927[9] and kindly given to this laboratory in 1934. Wild-type males from three further stocks were also used. They were derived by twice backcrossing FI hybrids between a wild-type aquarium race carrying the male sex-linked gene Maculatus and wild Trinidad stock from the St. Joseph, the Marianito, and the Maracas rivers to the wild stock, thus producing a genetically marked culture of seven-eighths wild genic composition. The sex-linked gene Maculatus was originally described by Winge[10] and, like Pauper, was obtained by us from Winge in 1934. It has been carried in mass culture since that time.

Finally, several males of Golden and Cream races were used, particularly in conditioning experiments. They were taken from races in which these autosomal factors had been combined with the sex-linked genes Pauper and Maculatus described above, and with a third such factor, Armatus, obtained from Danish domestic stock by Winge in 1922, described by him in 1927, and likewise obtained through his generosity in 1934.

Experimental Procedure and Results.—The experimental method was

essentially an elaboration of that described earlier.[1] Several aquaria of varying capacity were arranged to simulate the natural environment. Tanks varied in capacity from ten to twenty gallons. Into such tanks three or six females were introduced simultaneously, one or two each of Wild-type, Golden, and Cream stocks. The females selected all contained developing embryos (the normal condition in the wild species) and were taken directly from mass cultures of their own races. Males, singly in some cases and in groups in other tests, were then introduced. With one class of exceptions, such males were taken directly from stock cultures of their own races. Wherever possible in each test, females of the races corresponding to the males used were taken from precisely the same cultures as these males. This factor did not prove critical, however. Counts were then made of the number of gonopodial contacts of the introduced males with the females of their own and of the other two races on the first and subsequent days.

The exceptional experimental procedure noted above was that obtaining in experiments with "conditioned" or "reconditioned" males (tables 3 and 4). Here young males were raised in isolation until sexual maturity was attained, after which they were exposed for a period of a month or longer to a female of another race than their own. Such males were then introduced directly from the "conditioning" to the experimental tank. A few experiments were also undertaken in an attempt to "recondition" males already "conditioned" to females of races other than their own. Such individuals were transferred from the experimental tank to a new "conditioning" one, containing a single female of a race other than that to which they had previously been conditioned, immediately after the initial observations. After a period of exposure of a month or more to the new environment, they were returned to the experimental tank for further observation.

The results are shown in the following tables.

TABLE 1

WILD-TYPE MALES

SERIES A

Males from Trinidad (Maracas and Arima Rivers, Nariva Swamp, Maracas Beach) and Tobago (Delaford). Also males from backcrosses of Maculatus and wild stock from the St. Joseph, Marianito, Maracas, and Arima Rivers in Trinidad

	TOTAL MALES	WILD-TYPE	GOLD	CREAM	TOTAL
Wild stock	20	630	75	20	725
Backcrossed stock	61	134	11	5	150
Total	81	764	86	25	875

SERIES B

Males from mass culture of Pauper, Wild-type

MALES	WILD-TYPE	GOLD	CREAM	TOTAL	
2	190	26	4	220	
83	954	112	29	1095	Experimental Totals
	365	365	365	1095	Expected Totals on Basis of Chance Contacts

TABLE 2

MALES OF MACULATUS CREAM, ARMATUS CREAM, PAUPER GOLD, AND ARMATUS GOLD, TAKEN FROM MASS CULTURE

TYPE	TOTAL MALES	WILD-TYPE	GOLD	CREAM	TOTAL
Maculatus Cream	17	73	35	221	329
Armatus Cream	5	44	11	70	125
Pauper Gold	5	64	19	17	100
Armatus Gold	5	44	53	44	141

	TOTAL MALES	"CORRECT"		"INCORRECT"	TOTAL
Experimental Totals	32	363		332	695
Expected Totals on Basis of Chance Contacts		231.7		463.3	695

TABLE 3

"CONDITIONED" MALES, AS SHOWN

"Conditioned" to Wild-type

MALE TYPE	WILD-TYPE	GOLD	CREAM	TOTAL
Armatus, Cream	92	2	6	100
Armatus, Cream	91	0	9	100
Maculatus, Cream	87	7	2	96
Armatus, Gold	95	5	0	100

"Conditioned" to Gold

Armatus, Cream	9	87	10	106
Maculatus, Cream	104	14	7	125*
Armatus, Gold	5	73	23	101
Armatus, Wild-type	9	74	22	105

"Conditioned" to Cream

Armatus, Wild-type	6	6	13	25
Maculatus, Wild-type	8	8	84	100

	TOTAL MALES	"CORRECT"	"INCORRECT"	TOTAL
Experimental Totals	10	710	248	958
Expected Totals on Basis of Chance Contacts		319.4	638.6	958

* This exception was further tested with a diminutive wild-type female.   The results were confirmatory.

TABLE 4

"RECONDITIONED" MALES, AS SHOWN

TYPE OF MALE	INITIAL CONDITIONING	INITIAL REACTION		SECONDARY CONDITIONING	SECONDARY REACTION	
Armatus Cream	Female Gold, 11/11/49– 12/25/49	Wild-type: Gold: Cream:	9 87 10	Female Wild-type, 1/1/50– 2/22/50	Wild-type: Gold: Cream:	113 5 8
Armatus Cream	Female Wild-type, 10/14/49– 12/26/49	Wild-type: Gold: Cream:	92 2 6	Female Gold, 12/31/49– 2/19/50	Wild-type: Gold: Cream:	12 0 0
Armatus Cream	Female Gold, 11/11/49– 12/24/49	Wild-type: Gold: Cream:	5 73 23	Female Wild-type, 12/26/49– 2/19/50	Wild-type: Gold: Cream:	5 34 23

These three males were all closely similar, from the same mass culture of small population, inbred for fifteen years. They were treated in closely similar fashions. Yet it will be noticed that the second and third did not alter their original conditioning, while the first, which had followed a schedule closely similar to the others, did so alter markedly—an indication of the probable psychological variability of the males in this respect.

It is probable that males of *L. reticulatus* in mass cultures of their own race typically make a high proportion of gonopodial contacts with the larger females present. This is in all probability an instinctive behavior pattern, which may well be a result of natural selection. Every effort was made, therefore, to "match" the sizes of females used in each series as closely as possible. Exact matching was not feasible, but the effect of variable female size, as against variable color, was further reduced by using each set of females in experiments with differently conditioned males, predominant male choice then shifting from one race of females to another in each set.

Females were measured and weighed at the end of each series in which they were used. Since wild-type females tend to grow somewhat more rapidly in mixed culture than the recessive mutants, they were usually a little larger at the end of the experiments, even if carefully matched at the beginning. In order to be certain that the tendency to an over-all predominance for contacts with wild-type females, later referred to, was not in fact a size effect, some parallel test experiments were run in which the wild-type female used was conspicuously the smallest. The results were the same: males which showed a predominant tendency to contact the slightly larger wild-type female showed a similar predominance of contact with the markedly smaller one.

The weights and the lengths, measured from the snout to the base of the caudal peduncle, of the various series of females used are shown below.

Discussion.—In all, observations were made on the behavior of 125 wild-type Lebistes males from wild cultures taken from four representative localities in Trinidad, from aquarium-bred stocks and from backcross hybrids of these, and from aquarium stocks of several cultures of two races

differing from the wild type only in single autosomal recessives for body-coloration. A total of 2748 gonopodial contacts was recorded. Several interesting points emerged, which suggest possibly significant answers to the questions earlier raised.

It will be noticed from table 1 that wild-type males taken from mass culture exhibited remarkably good discrimination in differentiating between females of their own constitution and those of the two recessive races. This was true whether the males were actually wild individuals, individuals derived from wild cultures, of long inbred aquarium stocks, or were backcross hybrids between wild and laboratory stocks. Furthermore, this pronounced selectivity was exhibited toward any wild-type female, whether or not it was derived from the same specific stock as the wild-type male. Thus it was evidently the body color of the female which served as the basis of male discrimination.

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TABLE 5

MEASUREMENTS OF FEMALES USED IN COMPETITIVE TESTS

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FEMALE GROUP	WEIGHT, G.	LENGTH (SNOUT TO BASE OF CAUDAL FIN), MM.
I. Wild-type	...	28.0; 23 0 (Six females were used
Gold	...	25.0; 25.0 in this group)
Cream	...	25 0; 24 0
II. Wild-type	0.75	32.0
Gold	0.47	28.0
Cream	0 65	28 5
III. Wild-type	0.38	27.8
Gold	0.32	27.1
Cream	0 34	27.0
IV. Wild-type	0.21	23.5
Gold	0.07	22.9
Cream	0.10	22.9
V. Wild-type	0 75	32.0
Gold	0 47	28.0
Cream	0.65	28 5

The data recorded in table 2 indicate that males of Golden and Cream stocks taken from mass cultures of their own races exhibit a considerably better order of discrimination for females of those races than would be expected. It is of great interest, however, that their choice-pattern does not approach the perfection of that shown for wild-type females by wild-type males. This point was emphasized when observations were made on "conditioned" males, as shown in table 3. Of the ten conditioned males used, nine showed a choice of higher than expected accuracy for the females to which they had been conditioned, irrespective of their own genetic constitution. This appears to indicate rather definitely that the choice-pattern is at least in part a "learned" reaction, conditioned by the initial

exposure of the young male, and that in the absence of further conditioning factors it may be quite tenaciously retained.

In the presence of further conditioning factors, choice behavior may be modified much later in the life of the male, though the evidence of this is still incomplete. This is shown in table 4. The three males recorded there were of closely similar constitution. All were taken as immature fish from a small mass culture long inbred, and were given identical treatment throughout. It is of interest, therefore, that the first showed a pronounced modification of its original behavior, while the second and third adhered throughout to their initial patterns of conditioning.

A further point of interest emerges from these data. It will be noticed from table 3 that, although nine of the ten "conditioned" males showed an accuracy of choice higher than expectancy for the females to which they had been conditioned, this accuracy was not so good for males conditioned *away* from wild-type as in those conditioned *toward* wild-type. This is shown more effectively in table 6 in which all the data have been classified on the basis of conditioning toward or away from the wild-type.

TABLE 6

RELATIVE CHOICE EFFICIENCY OF MALES "CONDITIONED" TO WILD-TYPE AND TO OTHER BODY COLORS

"CONDITIONING"	"CORRECT"	"INCORRECT"	TOTAL	
Wild-type	1319	172	1491	Experiment
Wild-type	497	994	1491	Theoretical
Other	708	549	1257	Experiment
Other	419	838	1257	Theoretical

Total Contacts Recorded: 2748
Total Males Observed: 125

It will be seen that the differences are marked. It is clear from this data, and more evident in the data of table 4, that this difference cannot be attributed to a superior sensory endowment of the wild-type over the mutant male, for mutant males conditioned to wild-type responded as efficiently in their choice of wild-type females as did wild-type males conditioned to mutant females in their choice of the latter. It seems more probable that in addition to the conditioned, "learned" reaction, disposing the conditioned male to a predominance of contacts with females of the race to which it has been habituated, there is also another component, disposing the male, whatever its conditioning, to predominate in contacts with wild-type females. It is not clear at present what this component is.

Lastly, it seems evident from these experiments that complementary choice-patterns in the female, if they exist at all, cannot be of determining importance nor can a reaction-chain between male and female such as that

described for the stickleback have great significance in Lebistes. For it would not be expected that notable deviations of behavior-pattern would distinguish the females of the three color races of Lebistes used, nor could any such deviations in fact be observed. It seems extremely probable, therefore, that mate selection was made entirely by the male and that this discrimination was effected on the basis of vision, the body color or total body reflectivity of the female being of prime importance.

Although differences such as total body reflectivity are thus evidently determining for males confronted with choices between wild-type and mutant females of their own species, under other circumstances male discrimination may be accomplished with a high order of accuracy as a response to distinctions apparently much less conspicuous. This was early suggested by the ultimate high specificity of male discrimination between wild-type Lebistes females and the very similarly colored females of *M. parae* reported in the paper earlier cited.[1] In view of the present results, it became of interest to test it further. A choice situation was therefore set up for wild-type Lebistes males conditioned to wild-type Lebistes females between a female of *M. parae* on the one hand, and a mutant Lebistes female, which, as shown, would ordinarily be rejected in favor of a wild-type Lebistes female, on the other.

Sixteen wild-type Lebistes males taken from a mass culture of wild-type Maculatus stock twice backcrossed to wild Trinidad stock from the Arima River were given the choice between a typical female of *M. parae* and a Maculatus Cream female of Lebistes. The results on three days are shown in table 7. It will be seen that they agree closely with the choice patterns earlier reported between *M. parae* and wild-type Lebistes females. A rapid period of learning was exhibited, and within one week the choice, which originally favored *M. parae*, became 98% perfect for the Lebistes

TABLE 7

RELATIVE CHOICE EFFICIENCY OF WILD-TYPE LEBISTES MALES "CONDITIONED" TO WILD-TYPE FEMALES BETWEEN FEMALES OF *M. parae* AND MUTANT LEBISTES

Sixteen males of wild-type *Maculatus* stock, twice backcrossed to wild stock from the Arima River, Trinidad, taken from wild-type mass culture. Exposed to one wild female of *M. parae* and one female of Maculatus Cream, domestic stock, taken from mass culture of Maculatus Cream

ELAPSED TIME FROM INTRODUCTION	CONTACTS WITH *M. parae*	CONTACTS WITH MACULATUS CREAM
Day of introduction (*n*)	49	26
n + 1	2	23
n + 7	2	98

Length (snout to base of caudal fin) of *M. parae* female: 25 0 mm.

Length (snout to base of caudal fin) of *Lebistes* female: 24 0 mm.

Weight of *M. parae* female: 0.35 g.

Weight of *Lebistes* female: 0.31 g.

female, even though it was a mutant which would normally have been rejected, and was somewhat smaller and lighter in weight than the female of *M. parae*. Clearly neither size nor the rather close approximation of females of *M. parae* to the color pattern of wild-type female Lebistes (which appears to the human eye much closer than to the mutant Lebistes female) are as determining in choices of this sort as is conspecificity of the female. It is quite possible that minute behavioral factors in the female may be involved here. The predominance of contacts with the *M. parae* female during the first hours, however, strongly suggested in this case, as in the experiments earlier reported, that conditioning on the part of the male was most important, very probably involved with the mechanism of gonopodial contact.

Thus it would appear that in nature sexual discrimination of Lebistes for females of their own species, as contrasted with those of two superficially very similar species, is excellent, and that it is a contributing factor in maintaining specific isolation in the intimately sympatric associations of these species which occur in the brackish lagoons of Trinidad. It would appear that such discrimination is effected predominantly by the male, the female being essentially a passive agent. It is effected predominantly by visual means. This situation may throw suggestive light on the evolutionary significance of the brilliant and variable sex-associated color patterns so characteristic of Lebistes males, which should possibly be interpreted as primarily of intrasexual warning rather than of intersexual display value in natural selection.

The discrimination is in part a learned and "plastic" reaction, achieved through initial conditioning and subject to modification by later opposed conditioning, at least in certain individuals. In part, however, it appears to be innate and instinctive, and to have been established through natural selection. In nature, of course, these two components must reinforce one another to secure a predominance of contacts of male Lebistes with females of that species in the intimate sympatric associations in which it may exist.

Summary and Conclusions.--Earlier studies have indicated that males of *L. reticulatus* exhibit excellent powers of discrimination for females of their own species against those of two very similar related forms, *P. vivipara* and *M. parae*, which exist sympatrically with Lebistes in certain Trinidad waters. Such discrimination may constitute an important isolating mechanism for the species. The fact that the three species are largely intersterile in laboratory crosses and apparently hybridize very rarely in nature, however, makes a detailed examination of the mechanism of this discrimination difficult.

Experiments are described in which the accuracy of discrimination of Lebistes males between females of three races of the same species differing

in a single autosomal factor for body coloration is tested. This procedure is adapted to shed further light on certain aspects of the process. Observations were made in total on 141 males, involving counts of 2948 gonopodial contacts. The following conclusions seem justified:

1. Measuring the reaction of the male Lebistes to females of its own and other color races by the relative frequency of gonopodial contacts, a rather good discrimination by males for females of their own race can be demonstrated.

2. This discrimination obtains in males of all three autosomal color races of Lebistes used.

3. The discrimination is made predominantly or entirely by the male. There is no evidence of the existence of a chain of reactions between male and female, such as has been demonstrated for the stickleback.

4. The discrimination is predominantly or entirely visual.

5. In part, the discrimination is a "learned" reaction, which can be specifically modified by conditioning. The reaction of a male of any given race is decisively affected by its previous experience. Such discrimination is not a function of the genetic constitution of the individual male. Males which have been conditioned as young fish to one set of reactions can sometimes be later retrained to another, but the response to such retraining is variable.

6. Although males conditioned to wild-type females show marked preponderance of choice for wild-type females over the color mutants used, such mutants are preferred to wild females of *M. parue*, after an initial "learning" period. Thus the behavior pattern of such males follows closely that earlier reported in discrimination between wild females of Poecilia and Micropoecilia and of wild or wild-type Lebistes.

7. In part, the discrimination appears to be an innate, instinctive reaction, predisposing the male, whatever its own experience or genetic constitution, to make predominant contact with wild-type Lebistes females. Experiments of the type described could probably determine the relative importance of the learned and the instinctive components in the observed performance. In nature, the two components will be mutually reinforcing to produce a very efficient isolating mechanism for the species.

[1] Haskins, C. P., and Haskins, E. F., "The Role of Sexual Selection as an Isolating Mechanism in Three Species of Poeciliid Fishes," *Evolution*, 3, 160–169 (1949).

[2] Tinbergen, N., and Van Iersel, J. J. A., "Displacement Reactions in the Three-Spined Stickleback," *Ibid.*, 1 (1), 56 (1947).

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⁴ These body-color alleles to wild-type were described by Goodrich, *et al.*,⁵ Winge and Ditlevsen,⁶ Haskins and Druzba,⁷ and Haskins and Haskins.⁸

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⁶ Winge, Ø., and Ditlevsen, E., "Colour Inheritance and Sex Determination in *Lebistes*," *Heredity*, 1, 65 (1947).

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⁸ Haskins, C. P., and Haskins, E. F., "Albinism, a Semi-lethal Autosomal Mutation in *Lebistes reticulatus*," *Heredity*, 2, 251–262 (1948).

⁹ Winge, Ø., "The Location of Eighteen Genes in *Lebistes reticulatus*," *J. Genetics*, 18, 1-43 (1927).

¹⁰ Winge, Ø., "One-Sided Masculine and Sex-Linked Inheritance in *Lebistes reticulatus*," *Ibid.*, 12, 145–162 (1922).

ON THE MULTIPLICATION OF S-FUNCTIONS

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The functions which furnish the characters of the irreducible representations of the full linear group have been named, in honor of I. Schur, S-functions.   If m is any non-negative integer and if n is the dimension of the linear group there is associated with each partition of m into not more than n parts: $m = \lambda_1 + \lambda_2 + \ldots + \lambda_k$, $k \leqslant n$, $\lambda_1 \geqslant \lambda_2 \geqslant \ldots \geqslant \lambda_k$, an irreducible representation, of degree m, of the full linear group whose characters were determined by Frobenius.   Let $z_1, \ldots, z_n$ be the characteristic numbers of any element of the n-dimensional linear group; then the character $\{\lambda_1, \lambda_2, \ldots, \lambda_k\}$, of the representation in question, which corresponds to this element is the quotient of two n-rowed determinants whose elements are powers of the characteristic numbers $z_1, \ldots, z_n$.   The element in the pth row and qth column of the determinant in the numerator is z_q raised to the power $\lambda_p + n - p$ (it being understood that, if $k < n$, $\lambda_p = 0$ if $p > k$) and the element in the pth row and qth column of the determinant in the denominator is z_q raised to the power $n - p$ (so that the determinant in the denominator is the familiar Vandermonde determinant of the n numbers $z_1, \ldots, z_n$).   If, now, we have two representations of degrees m_1 and m_2, respectively, of the full linear group, the product of their characters, i.e., of the corresponding S-functions, furnishes the character of a representation of degree $m_1 + m_2$, in general reducible, of the full linear group (which representation is known as the Kronecker

product of the two given representations of the full linear group). It is with the problem of analyzing this product into its irreducible components that we concern ourselves in the present note. A rule furnishing this analysis was stated by Littlewood and Richardson in 1934[1] and proved by Robinson in 1938.[2] This rule involves the construction of appropriate diagrams of partitions of $m_1 + m_2$ from the diagrams of the given partitions of m_1 and m_2, respectively. A different method, based on a recurrence process, was given by us in 1937.[3] We point out now that a slight and obvious modification of Frobenius' formula for the characters of the irreducible representations of the full linear group furnishes, in what seems to us the simplest way, from the theoretical point of view, the desired analysis of the Kronecker product of any two of these irreducible representations. In the preparation of a table of products of Schur-functions, however, the recurrence process has definite advantages, particularly when m_1 and m_2 are large.

If we multiply the numerator of Frobenius' formula by Vandermonde's determinant (using row multiplication) we obtain an n-rowed determinant whose elements are sums of powers of the n characteristic numbers z_1, $\ldots$, z_n. On denoting by $s(p)$ the sum of the pth powers of these n numbers: $s(p) = z_1^p + z_2^p + \ldots + z_n^p$, $p = 0, 1, 2, \ldots$, the element in the pth row and qth column of the resulting product is $s(\lambda_p + 2n - p - q)$. The product of the denominator of Frobenius' formula by Vandermonde's determinant (i.e., the square of Vandermonde's determinant) is the n-rowed determinant of which the element in the pth row and qth column is $s(2n - p - q)$. On reversing the order of the columns in both numerator and denominator we have the following result:

The characters $\{\lambda_1, \ldots, \lambda_n\}$ of the irreducible representation, of degree m, of the full linear group which are associated with the partition $\lambda_1 \geqslant \lambda_2 \geqslant \ldots \geqslant \lambda_n \geqslant 0$ of m are the quotients of two n-rowed determinants; the element in the pth row and qth column of the determinant in the numerator is $s(\lambda_p + n - p + q - 1)$ while the element in the pth row and qth column of the determinant in the denominator is $s(n - p + q - 1)$.

The same argument shows that the characters of the Kronecker product of the two irreducible representations of the full linear group which are associated with the partitions $\lambda_1 + \ldots + \lambda_k = m_1, k \leqslant n, \mu_1 + \mu_2 + \ldots + \mu_j = m_2, j \leqslant n$, of m_1 and m_2, respectively, is the quotient of two n-rowed determinants whose elements are power sums of the n characteristic numbers $z_1, \ldots, z_n$. Of these the element in the pth row and qth column of the determinant in the denominator is again $s(n - p + q - 1)$ while the element in the pth row and qth column of the determinant in the numerator is $s(\lambda_p + \mu_{n-q+1} + n - p + q - 1)$. Thus all we have to do, in order to analyze the Kronecker product of the two given irreducible representations of the full linear group, is to analyze the determinant of which the element

in the pth row and qth column is $s(\lambda_p + \mu_{n-e+1} + n - p + q - 1)$ into a sum of determinants in each of which the element in the pth row and qth column is of the type $s(\lambda_p' + n - p + q - 1)$ where $\lambda_1' \geqslant \lambda_2' \geqslant \ldots \geqslant \lambda_n'$ is a partition, involving not more than n elements, of $m_1 + m_2$. To do this we consider the product $s(\lambda_1 + n - 1)s(\lambda_2 + n - 2) \ldots s(\lambda_n)$ and introduce n operators $x_1, \ldots, x_n$ which are such that x_q, $q = 1, 2, \ldots, n$, increases by 1 the argument of the qth of the n factors of this product. Thus the result of operating on $s(\lambda_1 + n - 1) s(\lambda_2 + n - 2) \ldots s(\lambda_n)$ by $x_1^{p_1} x_2^{p_2} \ldots x_n^{p_n}$ is $s(\lambda_1 + p_1 + n - 1) \ldots s(\lambda_n + p_n)$. Then the determinant of which the element in the pth row and qth column is $s(\lambda_p + \mu_{n-e+1} + n - p + q - 1)$ is the result of operating on $s(\lambda_1 + n - 1) \ldots s(\lambda_n)$ by the determinant of which the element in the pth row and qth column is x_p raised to the power $\mu_{n-e+1} + q - 1$. This determinant is the product of the Vandermonde determinant of the operators $x_1, \ldots, x_n$ by the Schur function of these operators which is associated with the partition $\mu_1 \geqslant \mu_2 \geqslant \ldots \geqslant \mu_j$ of m_2. The result of operating on $s(\lambda_1 + n - 1) \ldots s(\lambda_n)$ by the Vandermonde determinant of the operators $x_1, \ldots, x_n$ is the n-rowed determinant of which the element in the pth row and qth column is $s(\lambda_p + n - p + q - 1)$. All we have to do, then, to obtain the desired analysis of the Kronecker product of the two given irreducible representations of the full linear group is to write out $\{\mu_1, \ldots, \mu_j\}$ as a symmetric function (of degree m_2) of the operators $x_1, \ldots, x_n$ and to operate with this symmetric function on $\{\lambda_1, \ldots, \lambda_n\}$. The expression of $\{\mu_1, \ldots, \mu_j\}$ as a symmetric function of the operators $x_1, \ldots, x_n$ is readily carried out by the method explained on page 164 of my book *Theory of Group Representations.*[4] In carrying out the calculations it is well to take $n = m_1 + m_2$; this furnishes the analysis for all values of $n \geqslant m_1 + m_2$. If $n < m_1 + m_2$ we must discard all partitions of $m_1 + m_2$ which contain more than n non-zero elements. All disordered partitions must be rearranged in the usual way.

Example.—Analyze the square of the Schur-function $\{2, 1\}$. Here $m_1 = m_2 = 3$ and we take $n = 6$. Thus we write $\{2, 1\}$ in the form $\{2, 1, 0, 0, 0, 0\}$. From the table on page 154 of my book[4] we read off

$$\{2, 1\} = \sum_{i, j} x_i^2 x_j + 2 \sum_{i, j, k} x_i x_j x_k.$$

Hence

$\{2, 1\}\{2, 1\} = \{4, 2\} + \{4, 1^2\} + \{3^2\} + \{2^3\} - \{3, 1^3\} - \{2^2, 1^2\} -$
$2\{2, 1^4\} + 2\{3, 2, 1\} + 2\{3, 1^3\} + 2\{2^2, 1^2\} + 2\{2, 1^4\} = \{4, 2\} +$
$\{4, 1^2\} + \{3^2\} + 2\{3, 2, 1\} + \{3, 1^3\} + \{2^3\} + \{2^2, 1^2\}.$

This result is valid if $n \geqslant 4$; if $n = 3$ we must reject $\{3, 1^3\}$ and $\{2^2, 1^2\}$; if $n = 2$ we have $\{2, 1\}\{2, 1\} = \{4, 2\} + \{3^2\}$. The case $n = 1$ cannot arise since $\{2, 1\}$ has more than one non-zero element.

[1] Littlewood, D. E., and Richardson, A. R., *Phil. Trans. Roy. Soc.*, A233, 99–141 (1934).
[2] Robinson, G. de B., *Amer. Jr. Math.*, 60, 745–760 (1938).
[3] Murnaghan, F. D., *Ibid.*, 59, 437–488 (1937).
[4] Murnaghan, F. D., *Theory of Group Representations*, The Johns Hopkins Press (1938).

THE NON-FINITIZABILITY OF IMPREDICATIVE PRINCIPLES

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An impredicative class is a class definable only by reference to a totality to which the class itself belongs. Usual systems of set theory contain principles which provide us with infinitely many ways of generating such classes. We shall prove that we cannot in general replace these by finitely many, as we can in the case of predicative classes. In order to render the proof and what we prove clearer, we shall concentrate our attention on a rather special simple case. It will be obvious that other cases can be treated similarly.

Let L be the system determined by Bernays's axioms[1] I–III and Va. This is a system with finitely many axioms. Roughly speaking, in L we are assured that all finite classes obtained from the empty class by applying (any finite number of times) the operations of forming unit classes and sum classes are sets (membership-eligible classes), that every subclass of a set is a set, and that we have all predicative classes of these sets. Bernays has shown in detail that, by identifying natural numbers with certain sets of L, we can obtain the usual number theory in L.

For our purpose, we may describe L in the following manner. L contains the first-order predicate calculus (quantification theory) for one kind of variables X, Y, etc. (whose range consists of all classes) and an additional two-place predicate ϵ (membership). Since sets are merely classes which can be members of classes, variables ranging over sets can be introduced by contextual definitions such as: $(x)\phi x$ stands for $(X)((\exists Y)(X \epsilon Y) \supset \phi X)$. It follows immediately that if $(X)\phi X$ then $(x)\phi x$. Let us agree further that $X = Y$ stands for $(x)(x \epsilon X \equiv x \epsilon Y)$

The axioms of L can be stated thus:[2]

A1. $x = y \cdot x \epsilon Z \cdot \supset y \epsilon Z.$
A2. $(\exists x)(y)(y \epsilon x \equiv y \neq y).$
A3. $(\exists x)(y)(y \epsilon x \equiv \cdot y \epsilon z \vee y = w).$
A4. $(\exists x)(y)(y \epsilon x \equiv \cdot y \epsilon z \cdot y \epsilon X).$

A5. $(\exists B)(x)(x \in B \equiv (\exists y)(\exists z)(x = \langle y\ z\rangle \cdot y \in z)$.

A6. $(\exists B)(x)(x \in B \equiv (\exists y)(\exists z)(x = \langle y\ z\rangle \cdot z \in A)$.

A7. $(\exists B)(x)(x \in B \equiv (\exists y)(\langle y\ x\rangle \in A)$.

A8. $(\exists B)(x)(x \in B \equiv (\exists y)(\exists z)(\exists w)(x = \langle y\ z\ w\rangle \cdot \langle z\ w\ y\rangle \in A)$.

A9. $(\exists B)(x)(x \in B \equiv (\exists y)(\exists z)(\exists w)(x = \langle y\ z\ w\rangle \cdot \langle z\ y\ w\rangle \in A)$.

A10. $(\exists B)(x)(x \in B \equiv \cdot x \in A \cdot x \in C)$.

It is known that within L we can derive from A5–A10 the following general principle which covers them as special cases:

P1. If ϕ is normal, then $(\exists B)(x)(x \in B \equiv \phi(x, X_1, \ldots, X_k))$. In other words, the system determined by A1–A4 and the predicative principle P1 can be finitized.

Let us now consider the system Q determined by A1–A4 and, instead of P1, the following impredicative principle:[3]

P2. If ϕ is any propositional function, then

$$(\exists B)(x)(x \in B \equiv \phi(x, X_1, \ldots, X_k)).$$

We want to prove that if Q is consistent, we cannot derive P2 (with the help of A1–A4) from any finite number of its special cases.

The proof depends essentially on the fact[4] that the classes declared to exist by any (finite or infinite) number of special cases of P2 can be enumerated by treating these axioms as operations for generating new classes from given ones. It turns out that when we take only a finite number of special cases of P2, the enumeration of the classes declared to exist can be expressed in Q. So that we can define by the diagonal method a new class which is provided by P2 but does not occur in the enumeration. Consequently, no finite number of operations determined by special cases of P2 could generate all the cases of P2.

In order to exhibit the line of reasoning more clearly, we shall give within Q an enumeration[5] of the classes required by the axioms of L. Then it will be obvious that other systems can be treated in a similar fashion.

The following are six fundamental operations answering to the axioms A5–A10.

1.1. $F_0(X, Y) = E.$

 $F_1(X, Y) = V \times X.$

 $F_2(X, Y) = D(X).$

 $F_3(X, Y) = Cnv_2(X).$

 $F_4(X, Y) = Cnv_3(X).$

 $F_5(X, Y) = -(X + Y).$

Since we can develop number theory in Q, we can introduce variables m, n, etc., whose range consists of all natural numbers, and obtain ordinary

arithmetic functions. Consider now all the ordered triples $<m\ q\ k>$ ($m < 6$) of natural numbers and define in Q the following ordering relation S for them:

1.2. $<m\ q\ k>\ S\ <i\ j\ p>$ for $m, i < 6$: $q = j \cdot k = p \cdot m < i \cdot \vee$ max $\{g, k\} < \max\{j, p\} \vee \cdot \max\{q, k\} = \max\{j, p\} \cdot k < p \cdot \vee \cdot$ $\max\{g, k\} = \max\{j, p\} \cdot k = p \cdot q < j.$

With the help of S we can correlate the ordered triples $<m\ q\ k>$ ($m < 6$ with natural numbers by defining in Q (applying the usual technique o converting recursive definitions) a function J such that:

1.3. $J(0) = <0\ 0\ 0>;$
      $J(n') =$ (the ordered triple $<m\ q\ k>$ such that $(m < 6.(i)(j)(p)$
      $(J(n)\ S<i\ j\ p> \Longrightarrow \cdot<m\ q\ k> = <i\ j\ p> \vee <m\ q\ k>\ S<i\ j\ p>))$

Next we define in Q three functions $K_i'n$ ($i = 1, 2, 3$) which give the ith members of the triple $J(n)$:

1.4. $K_1'n = m$ for $(\exists k)(\exists j)(J(n) = <m\ k\ j>).$
      $K_2'n = k$ for $(\exists m)(\exists j)(J(n) = <m\ k\ j>).$
      $K_3'n = j$ for $(\exists m)(\exists k)(J(n) = <m\ k\ j>).$

Using these auxiliary notions, we can define in Q by induction a function F such that:[5]

1.5. $K_1'n = 0 \supset \vec{F}n = \vec{F_0}(F'K_2'n, \vec{F'}K_3'n);$
      . . . .
      $K_1'n = 5 \supset \vec{F}n = \vec{F_5}(F'K_2'n, \vec{F'}K_3'n).$

The formal definition in Q for this function F can be stated thus:

1.6. $G(m, B)$ for $D(B) = \{0, \ldots, m\} \cdot (k)(k \leqq m \supset : K_1'k = 0. \vec{B'}k =$ $F_0(\vec{B'}K_2'k, \vec{B'}K_3'k) \cdot \vee \cdot K_1'k = 1 \cdot \vec{B'}k = F_1(\vec{B'}K_2'k, \vec{B'}K_3'k) \cdot$ $\vee \cdot \ldots \cdot \vee \cdot K_1'k = 5 \cdot \vec{B'}k = F_5(\vec{B'}K_2'k, \vec{B'}K_3'k)).$

1.7. $<x\ n> \epsilon F$ or $x \epsilon \vec{F}n$ for $(\exists B)(G(n, B) \cdot <x\ n> \epsilon B).$

It should be easy to see that by applying induction on n we can prove in Q the six cases of 1.5 for the function defined in 1.7.

Then the enumeration of the classes which are declared to exist by the axioms of L is provided by the propositional function R:

1.8. $R(X, j)$ for $\vec{F}j = X.$

Hence, the classes declared to exist by the axioms of L are merely the classes X of Q such that $(\exists j)R(X, j)$.

Let us refer to a propositional function of Q as the translation of one of L if the former is obtained from the latter by restricting the class variables to range over classes X for which $(\exists j)R(X, j)$, or, in other words, by substituting $(X)((\exists j)R(X, j) \supset \phi X)$ for $(X)\phi X$. Then, by applying 1.5, we can prove in Q the translations A1'–A10' of the axioms A1–A10 of L. Moreover, it is also easy to see that the translation of any theorem of L is a theorem of Q. Hence, if the translation of a certain particular theorem of Q (which is of course also a propositional function of L) is not provable in Q, then the given theorem of Q is not a theorem of L.

Now we prove that if Q is consistent, then the following theorem of Q is not provable in L:

$$(\exists B)(n)(n \, \epsilon \, B \equiv n \; \epsilon \; \overrightarrow{F} n). \tag{1}$$

Thus, let M be the class defined by (1). If (1) were provable in L, then its translation would be provable in Q and we would be able to prove in Q: $(\exists j)R(M, j)$. However, by using the usual argument for Cantor's theorem, we can prove in Q: $\sim(\exists j)R(M, j)$. Thus, if $R(M, i)$, then, by 1.8 and (1), $(k)(k \, \epsilon \, \overrightarrow{F} i \equiv k \; \epsilon \; \overrightarrow{F} k)$. Therefore, we would have: $i \, \epsilon \, \overrightarrow{F} i \equiv i \; \epsilon \; \overrightarrow{F} i$, a contradiction. Hence, we have:[7]

THEOREM I. *If Q is consistent, then the theorem (1) of Q is not provable in L.*

It follows that the following theorem of Q is also not provable in L:

$$(\exists C)(x)(n)(<x \, n> \, \epsilon \, C \equiv (\exists B)(G(n, B) \cdot <x \, n> \, \epsilon \, B)). \tag{2}$$

This is so because, if (2) were provable in L, (1) would also be. However, for each given number m, we can prove in L a theorem:

$$(\exists C_m)(x)(n)(<x \, n> \, \epsilon \, C_m \equiv (\exists B)(G(m, B) \cdot$$
$$(y)(j)(j > m \supset <y j> \, \epsilon \, B) \cdot <x \, n> \, \epsilon \, B)). \tag{3}$$

This may be seen as follows. Obviously we can prove (3) when m is 0. If we can prove (3) for the case when m is $n - 1$, then we can use C_{n-1} and prove (3) for the case when m is n.

But in the sequence consisting of C_0, C_1, etc., each class contains all its predecessors, and the class defined by (2) is just the union (sum class) of them. Therefore, we get:

THEOREM II. *If Q is consistent, then there exists a monotone increasing sequence of classes such that each of these classes can be shown to exist in L but that their union cannot.*

Similarly, if L' is any system which contains as axioms, besides A1–A4, merely finitely many (say j) special cases of P2, then we can define relations

and functions and prove theorems which are similar to those given in 1.1-1.8, with j replacing 6. It will be a routine matter to construct and prove these definitions and theorems for any given system L'. The only definition which calls for some comment is the one answering to 1.6, which, unlike 1.6 itself, may contain bound class variables when expanded. However, since in Q we allow all propositional functions in defining classes, such a change will not affect[8] the proving of theorems that answer to I and II. We have:

THEOREM III. *If Q is consistent, then the theorem of Q which is related to L' as (1) is to L is not provable in L'.*

THEOREM IV. *If Q is consistent, then there exists a monotone increasing sequence of classes such that each of these classes can be shown to exist in L' but that their union cannot.*

Theorem IV seems to indicate a special kind of incompleteness for all systems which contain merely a finite number of special axioms of class formation.

From III, we obtain:

THEOREM V. *If Q is consistent, then P2 cannot be reduced (with the help of A1-A4) to any finite number of its special cases.*

We can prove a similar theorem for each system which contains P2 and, instead of A1-A4, certain other axioms serving similar purposes. Let us refer to[9] a system of Quine and two systems of the present author as the systems K, M and N. We have:

THEOREM VI. *If K (or M or N) is consistent, then P2 cannot be reduced to any finite number of its special cases.*

Before concluding, we note that by similar arguments, we can also prove the non-finitizability of the impredicative principles in systems containing classes of classes, etc. For example, let us take the system P used by Gödel.[10] If $T_n(n > 1)$ is the part of P which includes the variables and classes up to the type n but nothing higher, then we have:

THEOREM VII. *If T_n is consistent, then its principles of class formation (Komprehensionsaxiome) cannot be reduced to any finite number of their special cases.*

It follows immediately:

THEOREM VIII. *If the simple theory of types P is consistent, then its principles of class formation are not reducible to any finite number of their special cases.*

Similarly, in spite of the fact that we can obtain an extension of the Zermelo set theory with finitely many axioms by distinguishing between sets and classes, we can prove the following theorem:

THEOREM IX. *If Zermelo's set theory (with its axiom of infinity) is consistent, then the Aussonderungsaxiom in it cannot be reduced to any finite number of its special cases.*

We want to thank Professor Quine for valuable criticisms and suggestions.

[1] Bernays,.P., *J. Symbolic Logic*, **2**, 65–77 (1937); **6**, 1–17 (1941); **7**, 133–145 (1942).

[2] Unless otherwise explained, the notations and terminology will follow Gödel, K., *The Consistency of the Continuum Hypothesis*, Princeton, 1940. In particular, a normal propositional function will be a formula in which all bound variables are set variables or a formula equivalent to one such.

[3] This principle P2 has first been introduced by Quine, W. V. See his *Mathematical Logic*, Cambridge (Massachusetts), 1947 (2nd printing). P2 is his principle *202 on p. 162.

[4] This seems to have been first pointed out by Skolem, Th. (*Skrifter Norske Videnskaps-Akad. Oslo. I. Mat. Naturv. Klasse*, 1929, No. 4). Later R. Carnap also stressed this fact (*The Logical Syntax of Language*, London and New York, 1937, especially p. 267 ff). However, Carnap seems to overlook the circumstance that among the axioms he considers the Aussonderungsaxiom, unlike the others, actually embodies an infinite number of constructional operations.

As Skolem has pointed out (*Ibid.*), such an enumeration in general does not provide us with a consistency proof of the system under consideration, because we do not know whether we can divide all the pairs $<M\ N>$ (M, N being any two classes from the given enumerable totality) into two domains of ε-pairs and non-ε-pairs (according as M belongs to N or not) in such a way that all the theorems of the system will be satisfied. However, in the case of the system L where only certain sets (for which we can easily exhibit a model) and predicative classes of them are required by the axioms, the enumeration can actually be made in a natural fashion to provide an enumerable model (and therewith a consistency proof) for the system. An immediate consequence of this point has been used by J. Barkley Rosser and the present author (see their joint paper to appear soon in *J. Symbolic Logic*), and, independently, by A. Mostowski (in a talk referred to at bottom of p. 70, *J. Symbolic Logic*, **15** (1950)).

[5] This follows closely the construction in Gödel's monograph, op. cit., p. 35. However, our task is easier, because we have a fixed universe of sets all through our process of generating new classes.

[6] The class $\tilde{F}y$ is the class of all sets x such that $<x\ y>$ ε F. In order to avoid appeal to classes of classes, we are in 1.5–1.7 labeling the members instead of the classes themselves. A similar device has been employed under different circumstances by both Bernays (op. cit., p. 137) and Professor W. V. Quine (in his lectures, Spring, 1948).

[7] We observe incidentally that since L is known to have an enumerable model anyway, we could have used more direct arguments and proved in place of I a stronger theorem I′: (1) is not provable in L However, our present proof has the advantage of being applicable to other cases to be considered below.

[8] If L' contained infinitely many special cases of P2 as axioms, then we would not be able to obtain a definition answering to 1.6 because we would need an infinite disjunction (whatever that may mean) or variables ranging over classes of classes which transcend the system Q. This is also the reason why we cannot adapt the arguments for proving Theorem I to derive a contradiction from the axioms of Q.

[9] See, respectively, Quine's book, op. cit., p. 154; *J. Symbolic Logic*, **13**, 129–137 (1948); *Ibid.*, **15**, 25–32 (1950).

[10] It is a system of type theory with natural numbers as individuals. See Gödel, K., *Monatsh. Math. Phys.*, Vienna, **38**, 173–198 (1931).

ELECTROMAGNETIC PONDEROMOTIVE FORCES WITHIN MATERIAL BODIES

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Synopsis.—In classical electromagnetism the electrical force acting on a charged probe or small body is operationally defined as that force which must be added to the assumedly completely observable mechanical forces to restore otherwise failing classical particle mechanics. For a large body, rigid or non-rigid, lying in empty space, we may also define a total electromagnetic ponderomotive force as that force which added to the presumably observable total mechanical force restores otherwise failing mechanics. For such a body the total electromagnetic force thus operationally defined, may be determined by applying Maxwell's stress tensor in the empty space surrounding the body.

Very generally it is believed that specifically electromagnetic ponderomotive forces, volume and surface, exist within material bodies. Presumably these electromagnetic forces are to be defined by balancing properly with the mechanical stress tensor within the body.

One may attempt to define this mechanical stress tensor through the mechanical force required to keep the strain unchanged on making a cut along an element of surface within the body. However, in an electromagnetic field the force so obtained is not derivable from a tensor.

We may define as a possible electromagnetic stress tensor any tensor whose components are functions of the field vectors, E, D, H and B, and the charge and current densities ρ and i, and which in empty space, i.e., where $E = D$, $H = B$, $\rho = 0$, and $i = 0$ reduces to Maxwell's electromagnetic stress tensor. Then we define the associated mechanical stress tensor through the vector difference between the calculated electrical surface force for the sides of the cut, and the mechanical force observed there, this difference being derivable from a tensor.

These two largely arbitrary tensors meet all the requirements of mechanics and electromagnetism and no experiment can distinguish between the validities of the various sets of such possible tensors. There is then no physically significant uniquely definable volume and surface electromagnetic ponderomotive force within a material body.

For the interior of a material body in an electric field, a net force tensor may be defined which gives the observable net volume forces, and net forces on surfaces of discontinuity. This net force tensor may be calculated from the volume energy density when such volume energy density exists.

1. Definition of Total Electromagnetic Ponderomotive Force for Particles and Isolated Bodies.—Classical electromagnetism, the electromagnetism of macroscopic bodies, begins with the theory of electrical ponderomotive forces acting upon particles. It is found by experiment that under certain conditions the classical mechanics of these small macroscopic bodies fails. This classical mechanics asserts that for such a small body we must have

$$m\mathbf{a} = \mathbf{F}_m \tag{1}$$

where m is the mass, $\mathbf{a}$ the acceleration, relative to a suitable frame of reference and $\mathbf{F}_m$ the resultant "mechanical" force acting on the particle. $\mathbf{F}_m$ is asserted to be completely determinable from observations on the state of contiguous macroscopic bodies, as for example the twist of the suspension string, and the strain in the supporting bar of a Coulomb experiment, and from the gravitational action of remoter bodies. When electrical phenomena appear, equation (1) fails, and we *define* $\mathbf{F}_e$, the electromagnetic ponderomotive force on the particle, as that vector which restores (1) giving

$$m\mathbf{a} = \mathbf{F}_m + \mathbf{F}_e. \tag{2}$$

Experience then shows that $\mathbf{F}_e$ thus defined can, in a wide variety of cases, be expressed at any point in space empty except for the particle and the means for impressing the mechanical force, $\mathbf{F}_m$, by the equation

$$\mathbf{F}_e = q\left(\mathbf{E} + \frac{1}{c}[\mathbf{v} \times \mathbf{H}]\right) \tag{3}$$

where the scalar q depends on the previous preparation of the particle, and $\mathbf{E}$ and $\mathbf{H}$ are vectors in space independent of q and $\mathbf{v}$, the velocity of the particle. q, $\mathbf{E}$ and $\mathbf{H}$ are thus *defined* by equation (3) except for a multiplicative constant which may then be fixed by some arbitrarily chosen operational definition of the unit of q.

Thus these definitions of $\mathbf{F}_e$, q, $\mathbf{E}$ and $\mathbf{H}$ through the failure of mechanics, rest on the assumption that the mechanical forces which act on the particle are completely known, and that the means for exerting these mechanical forces may have no influence on the electromagnetic field, that is that $\mathbf{F}_e$, q, $\mathbf{E}$ and $\mathbf{H}$ will be independent of the particular means for effecting $\mathbf{F}_m$.

The wide variety of cases referred to above can be described generally as occurring when the dimensions of the small body or particle are small enough compared to the distances from other bodies, excluding the means for impressing the force $\mathbf{F}_m$, and when the small body is prepared so as to make q small enough.

For a large macroscopic body, rigid or non-rigid, mechanics also makes a general assertion, namely that

$$\frac{d}{dt}\mathbf{M} = \mathbf{F}_m \qquad (4)$$

where $\mathbf{M}$ is the total momentum of the body, and $\mathbf{F}_m$ is the total impressed mechanical force, which is determinable from the observations of the strains in contacting bodies, and the gravitational action of remoter bodies.

Again in the electromagnetic field (4) fails. Again, we may define a total electromagnetic ponderomotive force, $\mathbf{F}_e$, as that which restores (4) so that

$$\frac{d}{dt}\mathbf{M} = \mathbf{F}_m + \mathbf{F}_e, \qquad (5)$$

and again, for this definition to have meaning with content, we must assume that means are available for impressing $\mathbf{F}_m$ which do not affect the electromagnetic field, i.e., which give the same $\mathbf{F}_e$ independent of the means used for effecting the $\mathbf{F}_m$.

II. Limitation of This Paper to Steady Electric Fields.—While the author believes that the conclusions given in this paper are completely general, for the sake of brevity he will limit their exposition to the case of steady electric fields, and therefore to the case of bodies at rest, without electric currents.

III. Maxwell's Stress Tensor and Isolated Bodies.—Given a body, at rest, lying in empty space and in an electric field. The body is subjected to mechanical forces of the type referred to in Section I, which do not influence the field, and which give a total mechanical force $\mathbf{F}_m$ which may not be zero. $\mathbf{F}_e$ for this case, is then defined as $-\mathbf{F}_m$.

Surround the body by a closed surface S. $\mathbf{E}$ defined in Section I, is known at all points of S. Then, according to Maxwell (1), we have

$$\int\int_S \frac{1}{8\pi}(2\mathbf{E}\mathbf{E}\cdot d\mathbf{S} - \mathbf{E}^2\,d\mathbf{S}) = \mathbf{F}_e. \qquad (6)$$

We shall not take the space here to establish Maxwell's equation (6). A way of doing so would be to postulate certain additional properties of charged small bodies or macroscopic particles as given by experiment, and to show then that (6) holds if the only matter within the surface S is a finite number, though possibly very large, of such charged particles at rest. We then postulate that the Maxwell field theory is a successful "local action" theory, and that therefore (6) must hold irrespective of the actual nature of the material system within S.

We may not pass from the case of the system of charged particles to that of a continuous body by asserting that such a body may be regarded

as being in any meaningful sense a very dense system of charged macroscopic particles. If we proceed to cut a macroscopic larger body into smaller and smaller macroscopic parts, we do not get particles in the sense used in previous paragraphs, because the dimensions of these individual smaller parts do not become small compared to the distance to the neighboring smaller parts. It is only when we reach the microscopic world of electrons and nuclei that this aspect of particles is reached. But at this point macroscopic mechanics loses its meaning, and with it also classical electromagnetism. Certainly, according to our present ideas of the quantum mechanics which governs the microscopic particles, electrons and nuclei, there is no "mechanical force" acting on a microscopic particle, and therefore there is no electrical ponderomotive force as defined in Section I.

The integrand in (6) is a *linear* vector function of the vector element of surface dS. We may speak of it then as a force f_s acting on $|dS|$ which is derivable from a tensor, Maxwell's symmetric stress tensor for empty space.

IV. The Mechanical Stress Tensor?—How shall we now define the electrical ponderomotive force within continuous matter? It would seem that again the definition should be through the failure of ordinary mechanics. Presumably, in an electric field, the completely recognizable mechanical forces acting on any arbitrarily chosen continuous volume within the body will not balance according to the mechanics of continuous bodies, and we must invoke a volume electrical force F_s which we thereby define to restore this balance.

But what are these completely recognizable mechanical forces? In the mechanics of continuous bodies in the absence of electromagnetic fields, it is asserted that in addition to gravitational or inertial volume forces, there is acting on each element of the bounding surface, dS, of the volume, a force, $-f_m|dS|$ impressed by the contiguous matter,[1] and that this system of forces is derivable from a mechanical stress tensor, which is a function of the strains in the material. From the way this stress tensor is used in deriving the equations of mechanics, we may conclude that a meaningful operational definition of these mechanical forces is as follows.

Make a physical cut in the material along an element of surface. Introduce means for keeping the strains in the material on both sides of the cut the same as they were before the cut was made. Then the force introduced by these means is the force $-f_m|dS|$.

It is not assumed that the cut and the introduced means do not disturb the microstructure and micromechanics of the material. For example, in the case of a fluid the cut and means would cause molecules to be reflected which would otherwise pass through the geometric element of surface dS. It is assumed, however, that in spite of the change in the micromechanics, there is no change in the observable macromechanics.

Now in the case that there is an electric field, let us tentatively continue to define the mechanical forces acting on a volume within matter in the same way. Now, however, just as for the particles and isolated bodies of Section I, we must limit the means used for keeping the strains on the two sides of the cut unchanged by the cut, to such as will also leave the electric fields on the two sides of the cut un-
changed. Thus, for a dielectric fluid we may use as means, piston walls or heads made of very thin sheets of perfectly insulating material. But now, as we proceed to show, we run into the dilemma that the mechanical forces $-f_m|dS|$ thus found are not derivable from a tensor.

Consider a volume V, in a material, surrounded by a closed surface S^*. We now make a thin cut all along S^*, leaving a thin shell of empty space within which there will be an electric field E^*, figure 1. If the means used for impressing the forces $-f_m|dS|$ do not introduce charges, then E^* is related to the fields E and D within the material at S^* by the relations

FIGURE 1

$$E^* \cdot dS^* = D \cdot dS^* \qquad (7)$$

$$[E^* \times dS^*] = [E \times dS^*]. \qquad (8)$$

Now the volume V, bounded by the shell of empty space along S^* is an isolated body acted on by the purely mechanical external forces $-f_m|dS^*|$ in the sense of Section III, and we may apply the equation (6) giving

$$\iint_{S^*} \frac{1}{8\pi} (2E^*E^* \cdot dS^* - E^{*2} dS^*) = - \iint_{S^*} -f_m|dS^*| - F_m \qquad (9)$$

where presumably the right-hand member of (9) is the total electrical force acting on V, and F_m is the total volume distributed impressed mechanical force, or

$$-F_m - \iint_{S^*} -f_m|dS^*| = \iiint_V F_e \, dV \qquad (10)$$

where F_e is the presumably uniquely determinable ponderomotive electrical force per unit volume within V.
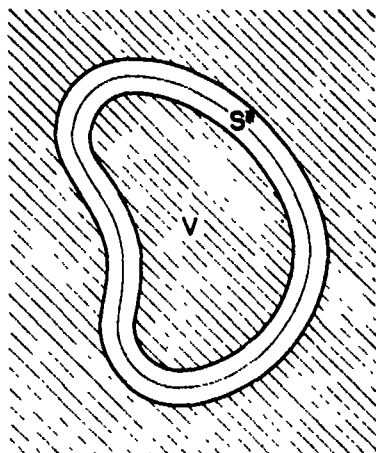
Now, if dS, unrelated to dS*, is an arbitrarily oriented element of surface within the fixed and unchanging shell of empty space, $\frac{1}{8\pi}(2\mathbf{E}^*\mathbf{E}^*\cdot d\mathbf{S} - \mathbf{E}^{*2}\,d\mathbf{S})$ is a linear vector function of dS. However, for the vector function of dS*, $\mathbf{f}_s|d\mathbf{S}^*| = \frac{1}{8\pi}(2\mathbf{E}^*\mathbf{E}^*\cdot d\mathbf{S}^* - \mathbf{E}^{*2}\,d\mathbf{S}^*)$, as the orientation of dS* is changed, the bounding walls of the cut must also change, and we may not conclude that $\mathbf{f}_s|d\mathbf{S}^*|$ is a *linear* vector function of dS*. In fact, applying equations (7) and (8) we see that $\mathbf{f}_s|d\mathbf{S}^*|$ is a cubic function of n the unit vector normal to dS*, and dS*, if D is not equal to E.

Hence, $\mathbf{f}_s|d\mathbf{S}^*|$ is not derivable from a tensor and $\int_{S^*}\int \mathbf{f}_s|d\mathbf{S}^*|$ is not equal to $\int_V\int\int\mathbf{F}_s\,dV$, where $\mathbf{F}_s$ is a vector independent of the shape of the volume V. Hence, by (9) $-\mathbf{f}_m|d\mathbf{S}^*|$ is also not derivable from a tensor, and our attempt to define the mechanical stress tensor through the forces which keep the strain unchanged on making a cut, has failed.
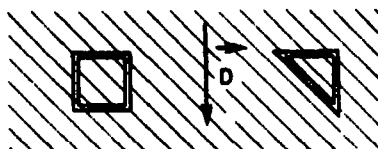
FIGURE 2

We may illustrate the foregoing development by a simple example. Consider a homogeneous dielectric material, with dielectric constant, k, very large, in a uniform field $\mathbf{D} = k\mathbf{F}$, figure 2. Consider a volume which is a unit cube with four faces parallel to D. Surround this cube with a cut making an empty shell space. In the two faces of the cut perpendicular to D, $\mathbf{E}^* = \mathbf{D}$. For these two faces the contribution to the component parallel to D of the Maxwell stress integral of (6) will be, respectively, $-\frac{1}{8\pi}\mathbf{D}^2$ and $+\frac{1}{8\pi}\mathbf{D}^2$. In the remaining four faces $\mathbf{E}^* = \mathbf{E} = \frac{1}{k}\mathbf{D}$ and is nearly zero. The component parallel to D of the Maxwell stress integral (9) is then zero, and therefore so also must be that component of the integral of $-\mathbf{f}_m|d\mathbf{S}^*|$ of (9) and (10).

But now consider the volume consisting of the half cube shown in figure 2 with one square face perpendicular to D, and the other square face and the two triangular faces parallel to D. In the cut along the square face perpendicular to D, $\mathbf{E}^* = \mathbf{D}$, and the contribution to the component parallel to D of the Maxwell stress integral of (6) is $-\frac{1}{8\pi}\mathbf{D}^2$.

For the three faces parallel to D, $\mathbf{E}^*$ is nearly zero and the contribution to the Maxwell stress integral is zero. For the remaining diagonal face, $\mathbf{E}^*$ will be nearly perpendicular to the face and of magnitude $|\mathbf{D}|/\sqrt{2}$. The contribution to the component parallel to D of the Maxwell stress.

integral is $+ \frac{1}{8\pi}\mathbf{D}^2/2$. The total Maxwell stress integral will then have a component parallel to $\mathbf{D}$ of magnitude $-\frac{1}{8\pi}\mathbf{D}^2/2$, and is not zero. Thus we see that the Maxwell stress integral, and that therefore also the integral of $-\mathbf{f}_m|d\mathbf{S}^*|$ depends on the shape and orientation of the volume considered, and that therefore the forces $\mathbf{f}_m|d\mathbf{S}^*|$ are not derivable from a tensor.

V. The Surface Electrical Ponderomotive Force.—The various formulae which have been proposed for the electrical ponderomotive force in matter, in the literature of classical electromagnetism, give surface forces at the bounding surfaces of material bodies, as well as volume forces within the bodies. This is because the proposed formulae for the volume force involve space derivatives of functions of the field vectors $\mathbf{D}$ and $\mathbf{E}$, and material parameters such as density and dielectric constant. At the boundaries of bodies the field vectors $\mathbf{D}$ and $\mathbf{E}$ are generally discontinuous, and also the material parameters as density, etc. In the mathematical application of the volume ponderomotive force formulae, the bounding surface is replaced by a thin layer in which $\mathbf{D}$, $\mathbf{E}$, and the material parameters vary continuously from their values within the body to their different values just outside the boundary. Application of the volume force formulae to this thin layer then leads to a surface force formula.

The appearance of these surface electrical forces suggests that there may be a way out of the dilemma presented by the fact that the mechanical forces which must be introduced in a cut to keep the strain unchanged, are not derivable from a tensor. We may say that on making the cut, we introduce new electrical ponderomotive forces, namely the surface forces which are related to and calculable from the volume electrical forces. We may say then that the mechanical forces $-\mathbf{f}_m|d\mathbf{S}^*|$ which are introduced into the cut must compensate for the surface electrical forces as well as take the place of the contiguous material in keeping the strain what it was before the cut was made. We might expect that after allowing for the surface electrical force, $\mathbf{f}_e|d\mathbf{S}^*|$, the remaining mechanical force, $(-\mathbf{f}_m + \mathbf{f}_e)|d\mathbf{S}^*|$, will be derivable from a tensor.

We are led then to a tentative circular kind of definition of the mechanical stress tensor and volume electrical ponderomotive force. The mechanical forces $-\mathbf{f}_m|d\mathbf{S}^*|$ in a cut are observed. Then the volume electrical force, $\mathbf{F}_e$ is such as leads to surface forces $\mathbf{f}_e|d\mathbf{S}^*|$ which make $(-\mathbf{f}_m + \mathbf{f}_e)|d\mathbf{S}^*|$ derivable from a tensor, and such that for any continuous volume, and bounding surface S^*,

$$\int\int_V\int \mathbf{F}_e\,dV + \int_{S^*}\int \mathbf{f}_e|d\mathbf{S}^*| = -\int_{S^*}\int -\mathbf{f}_m|d\mathbf{S}^*| - \mathbf{F}_m. \quad (11)$$

However, this does not lead to a unique electrical ponderomotive force and mechanical stress tensor.

VI.   *Non-uniqueness of the Electrical Ponderomotive Force and Mechanical Stress Tensor.*—We may readily see that infinitely many volume electrical forces, $\mathbf{F}_e$, with related surface force $\mathbf{f}_e | d\mathbf{S} |$ may be found which will satisfy (11) and which will make $(-\mathbf{f}_m + \mathbf{f}_e) | dS^* |$ derivable from a tensor. For this we may take any tensor whose components are functions of the field vectors, $\mathbf{E}$, $\mathbf{D}$, and the scalar charge density, ρ, and material parameters, such as density or dielectric constant, and which in empty space, where $\mathbf{E} = \mathbf{D}$, and $\rho = 0$, and the material parameters reduce to the appropriate values, becomes identical with Maxwell's electrical stress tensor. Then the divergence of this tensor gives a valid electrical volume force.

Maxwell's stress tensor itself, for empty space, by equation (6) has components,

$$7_{xx}^M = \frac{1}{8\pi}(\mathbf{E}_x^2 - \mathbf{E}_y^2 - \mathbf{E}_z^2)$$

$$7_{xy}^M = \frac{1}{8\pi}(2\mathbf{E}_x\mathbf{E}_y) \tag{12}$$

$$\text{etc.}$$

Now by (9) for any volume surrounded by an empty shell which follows the bounding surface S^*,

$$\int\int_{S^*} T_{ij}^M \, d\mathbf{S}_j^* = - \int\int_{S^*} -\mathbf{f}_{mi}| d\mathbf{S}^* | - \mathbf{F}_m \tag{13}$$

where T_{ij}^M refers to the empty space outside S^*, and the usual summation convention for repeated subscripts is implied.

We now consider any other tensor T_{ij} which reduces to T_{ij}^M in empty space. Then of course

$$\int\int_{S^*} T_{ij}^{(1)} \, d\mathbf{S}_j^* = \int\int_{S^*} 7_{ij}^M \, d\mathbf{S}_j^* = - \int\int_{S^*} \mathbf{f}_{mi}| d\mathbf{S}^* | - \mathbf{F}_m \tag{14}$$

where $T_{ij}^{(1)}$ refers to the values of T_{ij} outside S^*. We now apply Gauss's theorem to the first integral of (14) but take into account the fact that T_{ij} is discontinuous at S^*. We therefore have

$$\int\int_{S^*} T_{ij}^{(1)} \, dS_j = \int\int_{S^*} (T_{ij}^{(1)} - T_{ij}^{(2)}) \, dS_j^* + \int\int_{S^*} T_{ij}^{(2)} \, dS_j^*$$

$$= \int\int_{S^*} (T_{ij}^{(1)} - T_{ij}^{(2)}) \, dS_j^* + \int\int_V\int \frac{\partial T_{ij}}{\partial x_j} \, dV$$

$$= - \int\int_{S^*} -\mathbf{f}_{mi}| dS^* | - \mathbf{F}_m \tag{15}$$

where $T_{ij}^{(2)}$ refers to values of T_{ij} inside S^*.

Since T_{ij} is a tensor, $\dfrac{\partial T_{ij}}{\partial x_j}$ is the x_i component of a vector, which we shall now call the volume electric force, $\mathbf{F}_e$. $(T_{ij}^{(1)} - T_{ij}^{(2)})\, dS_j^*$ is the x_i component of a vector, if $\mathbf{dS}^*$ is fixed, and we call it the x_i component of $\mathbf{f}_e|dS^*|$, the surface electrical force related to $\mathbf{F}_e$. Then according to (15) we do have the necessary condition (11).

Furthermore, since V is an arbitrary volume, it follows that $(-\mathbf{f}_m + \mathbf{f}_e)|dS^*|$ is derivable from a tensor which we call the mechanical stress tensor, M_{ij}.

The non-uniqueness of the electrical and mechanical stress tensors, T_{ij} and M_{ij}, is evident from the method of their derivation. We may see directly, however, that they are not unique in their validity as follows. Let R_{ij} be any tensor function of the electrical field variables, the strain variables, and the electrical and mechanical parameters which we may choose to characterize the matter being examined. Then we may take as also valid electrical and mechanical stress tensors, $T_{ij}' = T_{ij} + R_{ij}$ and $M_{ij}' = M_{ij} - R_{ij}$. Since any experiment which can be performed can only determine the net force on a total body, or the net force applied to an external surface, and since these net forces are completely determinable from the sum of the two tensors, $T_{ij} + M_{ij}$, and since $T_{ij}' + M_{ij}' = T_{ij} + M_{ij}$, no experiment can distinguish between the validities of T_{ij}', M_{ij}', and T_{ij}, M_{ij}.

Throughout the literature of the subject, the electrical volume ponderomotive force is assumed to have unique meaning, although a meaningful operational definition is not given. One universal formula offered[2] is

$$\mathbf{F}_e = E(\rho + \rho') = \mathbf{E}\left(\frac{1}{4\pi}\operatorname{div}\mathbf{D} - \operatorname{div}\mathbf{P}\right) = \frac{1}{4\pi}\mathbf{E}\operatorname{div}\mathbf{E}, \quad (16)$$

where $\mathbf{P}$ is the polarization vector

Since

$$\iiint_V \frac{1}{4\pi}\mathbf{E}\operatorname{div}\mathbf{E}\, dV = \frac{1}{8\pi}\iint_S (2\mathbf{EE}\cdot\mathbf{dS} - E^2\,\mathbf{dS}) \quad (17)$$

we see that we have here one of the possible but not uniquely valid formulae as described in Section VI.

Another universal formula[1] is

$$\iiint_V \mathbf{F}_e\, dV = \iiint_V \mathbf{P}\cdot\nabla\mathbf{E}\, dV =$$

$$\frac{1}{8\pi}\iint_S 2\mathbf{ED}\cdot\mathbf{dS} - E^2\,\mathbf{dS} \quad (18)$$

again giving a tensor which agrees with Maxwell's in empty space.

For the special case of a material which has a dielectric constant, k, which is a function only of the density, τ, the formula

$$F_e = \frac{1}{8\pi}\left(-E^2 \operatorname{grad} k + \operatorname{grad} E^2 \tau \frac{dk}{d\tau}\right) \tag{19}$$

is frequently given.[4] Again we have that (19) reduces to

$$\iiint_V F_e\, dV = \frac{1}{8\pi}\iint_S 2ED\cdot dS - \left(E\cdot D - E^2\tau\frac{dk}{d\tau}\right)dS \tag{20}$$

and again on the right of (20) we have a tensor which reduces to Maxwell's stress tensor in empty space where $D = E$, and $\tau = 0$.
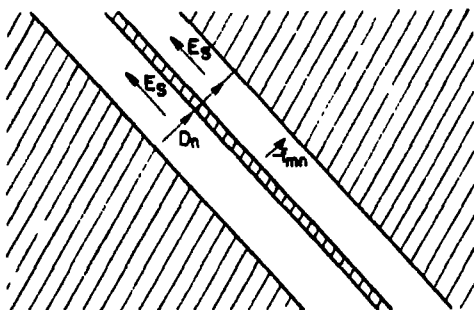
FIGURE 3

Expressions such as (19) are often derived[4] by assuming the existence of an energy density function, u, and equating the increase in the energy of a system, to the work done by applied force in a change of strain. It is clear, however, from the foregoing, that only net forces can be so determined, and that the designation of electrical parts and mechanical parts of such net forces is without meaning.

VII. *The Net Stress Tensor and Energy Density.*—It is clear that all the information which can be verified concerning the ponderomotive forces on a medium is contained in a knowledge of the field vectors E and D, and of the net force $-f_m|dS|$ at a cut, for all orientations of the cut, and at all points in the medium.

From equation (10) modified to include the application of net externally applied volume forces F, we have

$$\iint_{S^*} - \frac{1}{8\pi}(2E^*E^*\cdot dS^* - E^{*2}dS^*) + f_m|dS^*| = \iiint_V F\, dV. \tag{21}$$

It follows then that the integrand on the left of (21) defines a tensor which we call the net stress tensor.

If the matter in question has an electromechanical energy density function, u, then f_m and the net stress tensor may be determined therefrom, but they also have meaning independently of the existence of such a function.

As an example, take the case where there is an energy density u, which is a function of the electric field, E, or D, and the material density, τ, only.

Referring to figure 3, consider a cut, and in the cut, place a thin slab of material, subject to the same set of surface forces $-\mathbf{f}_m|dS|$ as the sides of the cut. Then in this slab, the field vectors, E and D, are the same as in the neighboring material.

Now give the slab a shear, slipping the one surface of the slab parallel to the other. Since the volume of the slab, and therefore also its density, and since also the field vectors are unchanged, then the energy of the slab is unchanged. The surface forces $\mathbf{f}_m$, therefore, do no work, during this shear, and therefore the force component parallel to the surface, $\mathbf{f}_{ms}$, is zero.

$$\mathbf{f}_{ms} = 0. \tag{22}$$

Now let the slab be expanded, by motion of its sides in the normal direction so that its volume per unit area is increased by ΔV. The increase in energy within the slab per unit area will be,

$$
\Delta(uV) = V\Delta u + u\Delta V = V\left(\frac{\partial u}{\partial \tau}\right)_{E_s, D_n} \frac{\Delta \tau}{\Delta V}\Delta V + u\Delta V
$$
$$
= \left(-\tau\left(\frac{\partial u}{\partial \tau}\right)_{E_s, D_n} + u\right)\Delta V \tag{23}
$$

where the subscripts E_s, D_n, indicate that E_s and D_n are to be kept constant during the differentiation of u with respect to τ.

The increase of energy per unit area in the empty space of the cut will be

$$-\frac{1}{8\pi}(E_s^2 + D_n^2)\ \Delta V.$$

D_s in the slab will be changed by the change in τ, and there will be energy per unit area of the cut supplied through the electric field given by

$$
\frac{1}{4\pi}\mathbf{E}_s\cdot\left(V\Delta\mathbf{D}_s + [\mathbf{D}_s - \mathbf{E}_s]\right)\Delta V = \frac{1}{4\pi}\left(-\tau\left[\frac{\partial D_s}{\partial \tau}\right]_{E_s, D_n} + D_s - E_s\right)\Delta V \tag{24}
$$

where of course, $\mathbf{D}_s$ is related to $\mathbf{E}_s$, $\mathbf{D}_n$, and τ by

$$\mathbf{E}_s = 4\pi\frac{\partial}{\partial D_s}(u[\mathbf{D}_s, \mathbf{D}_n, \tau]). \tag{25}$$

Equating the increase of slab energy (23) minus the loss of energy in the empty space, $\frac{1}{8\pi}(E_s^2 + D_n^2)\ \Delta V$ to the sum of the energy supplied electrically (24) and the work done mechanically, or $-\mathbf{f}_{mn}\Delta V$, we have

$$\left(-f_{mn} - \frac{1}{4\pi}\tau E_s\left[\frac{\partial D_s}{\partial \tau}\right]_{E_s, D_n} + \frac{1}{4\pi}E_s D_s - \frac{1}{4\pi}E_s^2\right)\Delta V =$$

$$\left[\left(-\tau\left[\frac{\partial u}{\partial \tau}\right]_{E_s, D_n} + u\right) - \frac{1}{8\pi}(E_s^2 + D_n^2)\right]\Delta V. \quad (26)$$

This, then, gives us

$$f_{mn} = + \tau\left(\frac{\partial u}{\partial \tau}\right)_{E_s, D_n} - u - \frac{1}{4\pi}\tau E_s\left[\frac{\partial D_s}{\partial \tau}\right]_{E_s, D_n} +$$

$$\frac{1}{8\pi}(D_n^2 - E_s^2 + 2E_s D_n). \quad (27)$$

To get the *net* mechanical volume force, **F**, which must be introduced to hold a given volume, V, within the medium in equilibrium, we then have (21) which we rewrite as

$$\iiint_V \mathbf{F}\,dV = - \iint_{S^0} \frac{1}{4\pi}\,ED \cdot d\mathbf{S}^* + \frac{1}{8\pi}(D_n^2 - E_s^2 -$$

$$2E_n D_n)d\mathbf{S}^* - f_{mn}d\mathbf{S}^* \quad (28)$$

Let us further specialize u to be of the form

$$u = \frac{1}{8\pi}k(\tau)E^2 + W(\tau) = \frac{1}{8\pi}\left(kE_s^2 + \frac{1}{k}D_n^2\right) + W = \frac{1}{8\pi}\mathbf{E}\cdot\mathbf{D} + W.$$

$$(29)$$

Then

$$\tau\left(\frac{\partial u}{\partial \tau}\right)_{E_s, D_n} = \frac{1}{8\pi}\left(E_s^2 - \frac{1}{k^2}D_n^2\right)\tau\frac{dk}{d\tau} + \tau\frac{dW}{d\tau} = \frac{1}{8\pi}(E_s^2 - E_n^2)\tau\frac{dk}{d\tau} +$$

$$\tau\frac{dW}{d\tau} \quad (30)$$

and

$$\tau\left(\frac{\partial D_s}{\partial \tau}\right)_{D_n, E_s} = \tau\frac{\partial}{\partial \tau}(kE_s) = E_s\tau\frac{dk}{d\tau}. \quad (31)$$

Substituting (31) and (30) into (27)

$$f_{mn} = -\frac{1}{8\pi}E^2\tau\frac{dk}{d\tau} + \tau\frac{dW}{d\tau} - W + \frac{1}{8\pi}(E_s D_s - E_n D_n - E_s^2 + D_n^2).$$

$$(32)$$

Substituting into (28)

$$-\iiint_V \mathbf{F}\, dV = \iint_{S^*} \frac{1}{8\pi}\,(2\mathbf{E}\mathbf{D}\cdot d\mathbf{S^*} - \mathbf{E}\cdot\mathbf{D}\, d\mathbf{S^*}) +$$

$$\left(\frac{1}{8\pi}\mathbf{E}^2\,\tau\,\frac{dk}{d\tau} - \tau\,\frac{dW}{d\tau} + W\right) d\mathbf{S^*}. \quad (33)$$

The integrand on the right of (33) is now a linear vector function of $d\mathbf{S^*}$, and defines a tensor, the negative of the net stress tensor. If we apply Gauss's theorem, we get

$$-\mathbf{F} = \rho\mathbf{E} - \frac{1}{8\pi}\,\mathbf{E}^2\,\mathrm{grad}\,k + \mathrm{grad}\,\frac{1}{8\pi}\,\mathbf{E}^2\,\tau\,\frac{dk}{d\tau} + \mathrm{grad}\left(W - \tau\,\frac{dW}{d\tau}\right)$$

$$(34)$$

where $\rho = \dfrac{1}{4\pi}\,\mathrm{div}\,\mathbf{D}$.

Equation (34) gives the negative of the net volume force which is impressed on the material to hold it in equilibrium. In various places, the first term, the sum of the first two terms and the sum of the first three terms are designated, respectively, as the electric volume force.

[1] In subsequent integrations over a closed surface enclosing matter, the direction of $d\mathbf{S}$ will be that of the outer normal. This is the reason for the negative sign used here.

[2] Richardson, *The Electron Theory of Matter*, Cambridge University Press, p. 206, 1914.

[3] Page and Adams, *Principles of Electricity*, D. Van Nostrand, New York, 15th Printing, pp. 45-49.

[4] Stratton's *Electromagnetic Theory*, McGraw-Hill, 1941, p. 139. A number of early references are given on pp. 146 and 150

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INFRA-RED BANDS IN THE SPECTRUM OF NH₃

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Measurements made in this laboratory attempting to establish whether or not ammonia was a component of the earth's atmosphere required the remeasurement in the laboratory of the fundamental bands in the ammonia spectrum. It was found, notably in the bands ν_2 and ν_4, that the spectra were appreciably better resolved than in earlier attempts and it has therefore seemed of interest to look at these again with some care. Measure-

ments have been completed on the four fundamental bands and as well on the low frequency difference band originating with molecules in the excited state $\nu_2(1^-)$ making a transition to the state $2\nu_2(2^+)$. A portion of this band had been seen earlier by Sheng, Barker and Dennison,[1] but an interesting portion of the band was hidden by the intense carbon dioxide fundamental band overlapping this region. A certain amount of water vapor falsification was also noted in this region. It has seemed desirable to make a preliminary report on these measurements at this time because it will demand considerable time to complete the calculations on the molecular constants which these data seem to warrant.

The measurements were made in all cases, except on the 10 μ band, using a vacuum grating spectrograph described elsewhere[2] so that the falsifying effects due to water vapor and carbon dioxide could be eliminated. The samples of gas were also carefully dried. One method which proved
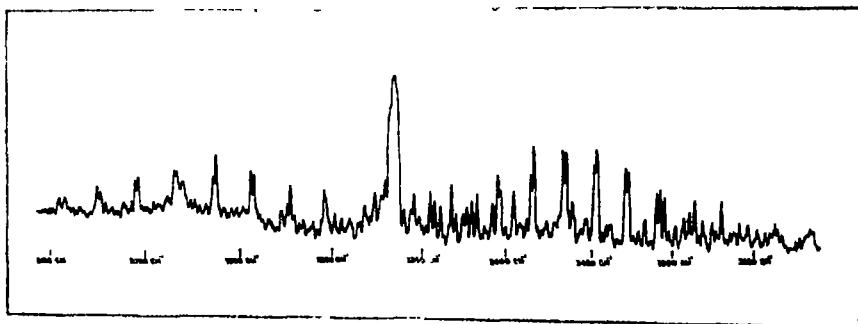
FIGURE 1

ν_4 in the infra-red spectrum of NH_3.

particularly efficacious was to place a small amount of magnesium nitride in the absorption cell, any residual water vapor which might be present being thus absorbed and NH_3 being liberated according to the reaction $6H_2O + Mg_3N_2 \rightarrow 2NH_3 + 3Mg(OH)_2$.

Figure 1 shows the absorption pattern of the perpendicular band ν_4 studied first by Barker.[3] Our pattern verifies in general that shown by Barker. Narrower slits could be used in our experiment because the effects of atmospheric water vapor could be eliminated and considerably better resolution has been achieved. The pattern of Barker could substantially be obtained by operating our spectrometer with somewhat widened slits. A complete analysis of this band has not been made although many of the characteristics which are to be expected have been spotted. This region was studied using an echellette replica grating with 3600 lines per inch and with effective slit widths less than 0.5 cm.$^{-1}$.
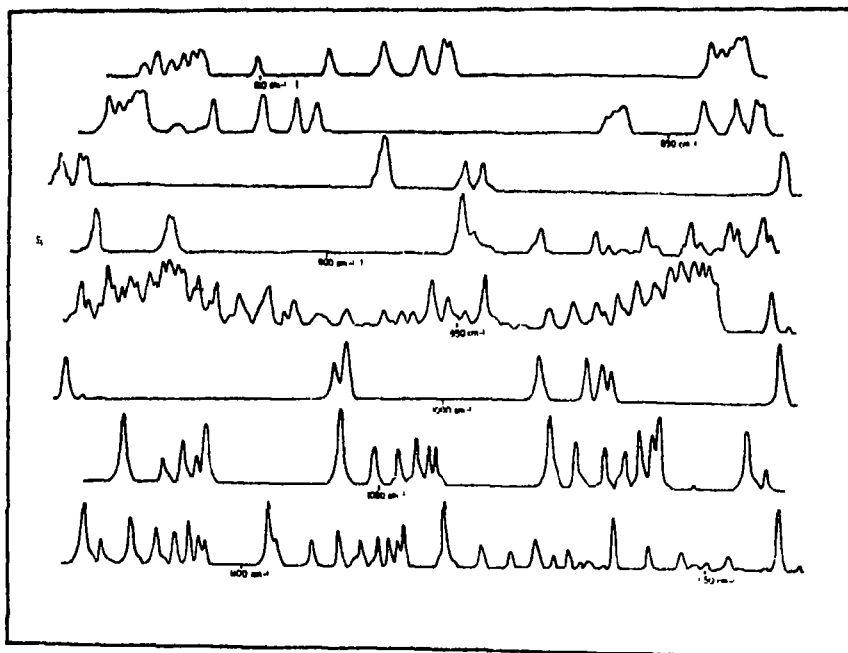
FIGURE 2

Transitions ν_2 $(0^- \rightarrow 1^+)$ and ν_2 $(0^+ \rightarrow 1^-)$ in the infra-red spectrum of NH$_3$.

NH$_3$
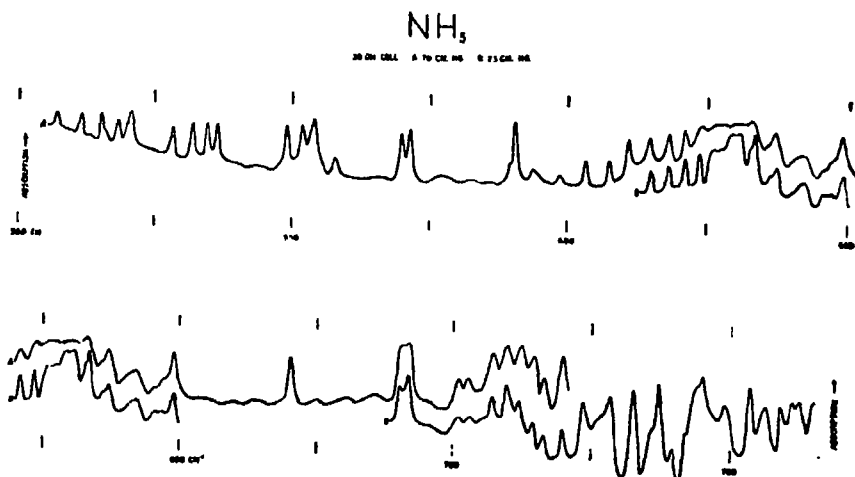
FIGURE 3

The transition ν_2 $(1^- \rightarrow 2^+)$ in the infra-red spectrum of NH$_3$.

Figure 2 shows the absorption pattern of the famous double band in ammonia known as ν_2 (i.e., $\nu_2(0^- \to 1^+)$ and $\nu_2(0^+ \to 1^-)$). This vibration is the one simulated by a relative motion of the N atom normal to the plane of the H_3 triangle. The vibration levels occur in pairs because of the fact that two equilibrium positions of the N atom relative to the H_3 triangle must exist, the intervals between these pairs increasing rapidly with vibration quantum number. Two overlapping parallel bands will result for this reason rather than one. Here also somewhat better resolution has been achieved than reported elsewhere. The measurements were made using the same replica echellette grating with 3600 lines per inch referred to earlier. The slit widths were about 0.25 cm.$^{-1}$.
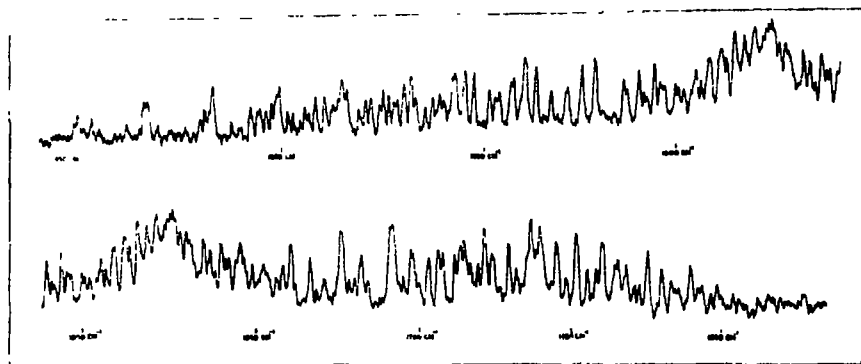
FIGURE 4

ν_1 and ν_3 in the infra-red spectrum of NH_3.

Evidently since the intervals between pairs of levels in this vibration state intervals with vibration quantum number a similar double band should arise with molecules already populating the states $\nu_2(1^+)$ and $\nu_2(1^-)$ making transitions to the states $2\nu_2(2^-)$ and $2\nu_2(2^+)$. Indeed a portion of the transition $\nu_2(1^- \to 2^+)$ has already been measured by Sheng, Barker and Dennison.[1] It has been possible for us to study the entire band because, by evacuating the spectrograph, we were able to eliminate the interference due to atmospheric carbon dioxide and water vapor. The component band $\nu_2(1^- \to 2^+)$ is shown in figure 3. At the high frequency end interference with the band ν_3 may be seen. The other components, $\nu_2(1^+ \to 2^-)$, may be expected to lie close to 970 cm.$^{-1}$ and would, therefore, lie on top of the fundamental ν_2. It has not been possible to identify this component because of the interference by the much more intense band ν_2. The band $\nu_2(1^+ \to 2^-)$ was measured using an original echellette grating ruled at the University of Michigan by Barker with 1200 lines per inch. No separations less than about 1 cm.$^{-1}$ were observed.

The two bands shown in figures 2 and 3 are probably the ones of most immediate interest. As suggested by Sheng, Barker and Dennison, each J transition in the P and R branches is split into its K-components. The P branch of the low frequency component of ν_2 (i.e., $\nu_2(0^{-1} \rightarrow 1^+)$) has been resolved sufficiently so that all the components may be seen. As indicated by Sheng, Barker and Dennison the $P(1)$ line is absent, the $P(2)$ line is double, the $P(3)$ line is double, the $P(4)$ line is quadruple, the $P(5)$ line is quadruple, etc. This may be seen to be the case in figure 2. Similarly it may be shown that the $R(0)$ and $R(1)$ lines should be single, the $R(2)$ and $R(3)$ lines, triple, the $R(5)$ and $R(6)$ lines, quintuplets, etc. Our spectrogram shows the $R(1)$, $R(3)$ and $R(5)$ lines to have the number of components predicted, but in the case of the $R(2)$, $R(4)$, $R(6)$ lines the high frequency component is not resolved.

A comparable resolution has been achieved in the band $\nu_2(1^- \rightarrow 2^+)$ to that obtained for ν_2. The $P(1)$ line is absent; the $P(2)$ line is an unresolved doublet; the $P(3)$ line is double as predicted; the $P(4)$ line appears triple, the fourth component remaining unresolved; etc. On the high frequency side the first few R lines may be distinguished, but overlapping with ν_2 quickly makes identification difficult.

For the sake of completeness figure 4 is included which shows the parallel band ν_1 and the overlapping band ν_3. It is essentially like the work reported by Hardy and Dennison[4] except that some of the details of the perpendicular band are different, probably because the falsification due to water vapor could here be eliminated.

A more complete discussion of this work will be published elsewhere when the calculations on the data have been completed and a more complete analysis has been carried out.

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NATIONAL ACADEMY OF SCIENCES
CONFERENCE ON THE ULTRACENTRIFUGE*

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On June 13–16, 1949, a conference on the ultracentrifuge, sponsored by the National Academy of Sciences and organized by D. A. MacInnes, was held at the Ram's Head Inn, Shelter Island, New York. The participants were W. J. Archibald, J. W. Beams, P. Ecker, L. J. Gosting, H. S. Harned, C. W. Hiatt, W. Kauzmann, G. Kegeles, L. G. Longsworth, D. A. Mac-Innes, T. L. McMeekin, D. H. Moore, J. L. Oncley, B. B. Owen, K. O. Pedersen, M. L. Randolph, T. Shedlovsky, R. Trambarulo, M. Wales, and J. W. Williams.

The following is a brief account of the topics that were discussed at the conference. It is hoped that this report will serve to bring to the attention of all workers in the field some of the outstanding problems of the ultracentrifuge and to stimulate efforts toward their solution. As reporter for the conference the author submitted a preliminary draft of this manuscript to the participants and gratefully acknowledges their generous cooperation in the correction of errors, and the suggestion of the many revisions that are incorporated in the following account.

Introduction.—The discussion at the conference on the ultracentrifuge was organized, in large measure, around each of the terms that appear in the expression for the determination of molecular weight with the aid of the sedimentation velocity method.[1] In this expression, which is:

$$M = \frac{RTs}{D(1 - \rho v)} \tag{1}$$

s is the sedimentation velocity, D the diffusion coefficient, v the partial specific volume of the material being studied and ρ the density of the solution. The meaning and experimental evaluation of each of these quantities were considered, as well as the temperature, T, of the measurements. Only the gas constant, R, was taken for granted.

Although the principal use of the ultracentrifuge has been in the determination of the molecular weight and homogeneity of proteins an attempt was made to avoid too much discussion of the many problems connected with proteins as such. Thus in the session devoted to diffusion, and again in the one concerned with density and partial specific volume, considerable reference to work on salts was made since these materials have been used in testing and improving the experimental procedures. A most profitable period was devoted to a consideration of the problems that are peculiar to

the equilibrium ultracentrifuge. In the consideration of the sedimentation constant much of the discussion centered around centrifuge design since it is in the construction of high-speed machines capable of sedimenting small molecules that the serious engineering problems arise.

Centrifuge Design and the Sedimentation Constant.—The discussion of the relative merits of the contemporary air-, oil- and motor-driven[2] centrifuges was overshadowed by the glimpses of the centrifuges of the future given by J. W. Beams.[3] Magnetically supported and driven, the rotors of these machines spin about a vertical axis in sealed containers that permit the attainment and preservation of the best vacuum possible. Consequently the frictional losses are so low that the rotor can be kept, with negligible temperature change, in synchronism with a crystal-controlled rotating magnetic field. Owing to the radial symmetry of both the supporting magnetic field and the steel core of the rotor on which it acts, heating effects from this source are also negligible. Although the supporting field stabilizes the rotor axis on the magnetic axis with respect to radial displacements, automatic compensation for vertical displacements is achieved by feed-back control of the current through the supporting coil with the aid of a small secondary winding, placed beneath the rotor, whose inductance is influenced by the proximity of that body.

The most serious problem in connection with these machines, for which an entirely satisfactory solution has not yet been obtained, is that of acceleration without excessive heating of the rotor. In order to accelerate, some slippage between this element and the rotating field must occur and this lack of synchronism results in the generation of heat that can be dissipated from the rotor only by radiation. Work is in progress directed toward the solution of this problem.

Research at the University of Virginia has also included preliminary tests of an interference method for the detection and recording of refractive index changes in the centrifuge cell. As in the Michelson and Mach-Zehnder interferometer a beam of collimated light is split with the aid of a half-silvered mirror, one beam being passed through the centrifuge cell, the other through the reference cell at the opposite end of the rotor diameter, and the two beams then recombined before entering a telescope focused on the cells. In order to have interfering rays pass through levels in the two cells that are equidistant from the axis of rotation it is necessary to reverse the direction of one of the beams. Otherwise the ray through the top of one cell interferes with that through the bottom of the other cell. Moreover, in the absence of a synchronized shutter the alternate passage of both cells through each beam leads to the superposition of a reversed fringe pattern upon the one that is obtained with the centrifuge and reference cells interchanged.

The sensitivity of the interference method has served to focus attention

upon the distortion of the cells that occurs at high speeds. Owing to its high compression strength, crystal quartz is used for the windows, and the faces of both these and the quartz cell body are ground to a flatness of $1/_{10}$ wave prior to being cemented together. Although an assembled cell is "floated" in the rotor in a thin Lucite sleeve the fringes are distorted at high speeds even with solvent in both cells. This observation emphasizes the difficulties of building cells that will have a common distortion under large stresses. As will be noted later in this report many of these difficulties disappear at the lower speeds that are characteristic of the equilibrium ultracentrifuge.

Although it is probable that the magnetically supported and driven centrifuge will eventually supplant contemporary instruments, development of the oil- and air-driven machines continues. In the case of the air-driven type, Beams' method of controlling the rotor speed has proved to be quite effective. In this method[4] an armature is attached to the driving rotor that, together with its field circuit, absorbs the excess driving energy as the speed approaches the resonance frequency of that circuit. Moreover, progress in the solution of the problem of measuring the rotor temperature *in situ* with the aid of thermistors was reported by Hiatt and Ecker.[5]

Resolving Power.—In the conventional sedimentation velocity method the resolving power of the instrument depends, in part, on the radial depth of the cell, and the strength of the rotor materials places restrictions on this depth. In the case of a mixture of two proteins of similar sedimentation constant, for example, where both would be thrown to the bottom of the cell before appreciable separation occurred, resolution can still be achieved if the proteins can be made to sediment (a) against a countercurrent of solution moving toward the axis of rotation or (b) against electric migration in the same direction. Progress with the migration procedure was reported by Randolph, who started this work at Virginia but is now carrying it forward at Tulane. Moreover, the experience gained in the construction of the multi-channelled centrifuge cells that are required in this work will doubtless prove of value in the development of such cells for use with the interference optical methods requiring a reference channel near that in which sedimentation occurs.

Diffusion.—The discussion, at the conference, of methods for the determination of diffusion coefficients served a dual purpose. Not only is a knowledge of D essential for a determination of M, equation 1, by the sedimentation velocity method but it also appears that the interferometric optical methods that are being adapted for diffusion studies will also find an application in centrifuge work. In fact progress in this direction may be most rapid if the various optical arrangements are tested on the concentration gradients that arise in diffusion before the mechanically difficult adaptation to the centrifuge is attempted.

Until recently the Lamm scale method has been almost universally used for the measurement of the refractive index gradients that arise in the free diffusion of proteins and related materials. The recent adaptation of the Gouy[6] and of the Rayleigh fringes to diffusion studies indicates, however, the trend to interference methods for this purpose. Although the optical methods are applicable to either electrolytes or non-electrolytes, in the case of dilute salt solutions sufficient precision has not yet been achieved to permit direct comparison with diffusion coefficients computed from electric mobilities with the aid of the Onsager-Fuoss theory. Using a conductance method the recent work of Harned[7] and the Yale group has, however, provided us with this essential control. Although not applicable to proteins the validity of their method has been indicated by the agreement that is obtained with the theory in the case of dilute solutions of, for example, potassium chloride. Moreover, in the case of more concentrated solutions of this salt the agreement between the results with the conductance method and those obtained by Gosting at the Rockefeller Institute with the Gouy method validates, in turn, this optical procedure.

In spite of its inherent precision the Gouy method has the limitation that the theory is restricted to the diffusion of an ideal solute, or to a mixture of such solutes, and that the fringes indicate the magnitude of the gradients in the diffusion channel but not their positions. Without abandoning this method investigators are, nevertheless, exploring the possibilities of other interference methods. Thus the adaptation of the Rayleigh fringes suggested by Philpot and Cook[8] holds promise if satisfactorily corrected cylinder lenses can be obtained. In common with the more conventional interference methods this gives the refractive index as a function of the height in the diffusion column.

It is well to bear in mind, however, that, in contrast with conventional interferometry, a layer of solution in the diffusing boundary deflects the ray as well as retarding it. If errors from this source are to be minimized compensation for the deflection should be achieved before the ray is allowed to interfere with that through the reference channel. Moreover, in the case of the Rayleigh fringes, if the resolving power of the photographic emulsion is not to be a limiting factor and if enlargement of the fringe system is to be avoided, lenses of rather long focal length are required. The objective of most investigators in this field is the development of an optical method, probably an interferometric one, that will give both the refractive index and its gradient as a function of the height in the diffusing boundary.

Diffusion of Proteins.—Owing to the presence of neutral and buffer salts and to uncertainty as to homogeneity the study of protein diffusion is a more difficult problem than that of low molecular weight substances. In order to minimize charge effects the protein is usually examined with a

buffered salt solution at the isoelectric pH of the protein as "solvent."
Thus the system contains a minimum of two solutes. Since solution and
solvent are prepared by dialysis of one against the other the constancy of
the chemical potential of the dialyzable component across the initial
boundary is thereby assured. It is not obvious, however, that this con-
stancy is preserved as the protein diffuses and precise work may reveal
coupling of the salt and protein transport processes. Moreover, with a
substance whose diffusion coefficient is concentration dependent it is es-
sential that the "solvent" contain the solute at a concentration only
slightly less than that of the underlying solution, at least if the treatment
of the optical data assumes ideal diffusion. Although the coefficients of
most proteins do not change rapidly with the concentration Pedersen re-
ported that even for these materials the use, at Upsala, of differential dif-
fusion had improved materially the reproducibility of the measurements.

As was emphasized by Kegeles when two solutes are present greater
care must be exercised in the manipulation of the solution than in the case
of a single solute. For example, with a single solute a change of concentra-
tion due to evaporation, say, produces a much less serious error than in the
case of a protein dissolved in a buffer solution where such evaporation
would introduce, across the initial boundary, a gradient of chemical poten-
tial for the salt in addition to that for the protein.

Density and Specific Volume.—In the centrifuge equation, equation 1,
the density volume product, ρv, has a value of approximately 0.75 for
many proteins. This product is, however, subtracted from unity and if the
term in parentheses is to have the same precision as s and D, ρv should be
known with a precision three times as great.

In spite of the fact that pycnometer methods have long been considered
as adequate for the determination of density, MacInnes has recently de-
veloped further for this purpose the float method of Lamb and Lee[9] in .
which the third and fourth decimals in the weight of solution displaced by a
60-ml. float are determined magnetically. Although applied thus far only
to salt solutions it combines speed with an uncertainty in ρ of a very few
parts per million and may facilitate the evaluation of the partial specific
volume term in the case of proteins.

Although little difficulty is experienced in the determination of the
density, ρ, of the solution with the desired accuracy, at atmospheric pres-
sure at least, this is not true of the partial specific volume v since here a
knowledge of the protein concentration is required. The uncertainties
that limit the precision of v are not only in the measurement of solution
densities but in the determination of concentration, i.e., uncertainty as to
the homogeneity and reproducibility of the protein and lack of knowledge
as to the moisture content of the sample or the nitrogen factor if the solu-
tion is analyzed by the micro-Kjeldahl method. The recent observation

by McMeekin[10] that the volumes of many proteins are the sums of the volumes of their amino acid constituents should serve as a useful guide in this work as the compositions of additional proteins become available.

Following considerable discussion of the question it was finally agreed that, in the equilibrium method at least, the density, ρ, is that of the solution in which the particle is suspended, not the density of the solvent, and that v is the partial, and not the apparent, volume of the protein. In the case of the sedimentation velocity method, on the other hand, the only safe procedure appears to be that used in Upsala in which the values of s, D, and v, equation 1, are extrapolated to zero concentration of the protein after correction of s and D for the viscosity effects due to the non-sedimenting solutes. The density, ρ, is then that of the pure solvent at the temperature, T, account being taken of the pressures in the cell. The necessity for this procedure arises from the probability that at finite concentrations the value of the friction coefficient in sedimentation is not identical with its value in diffusion. The need for theory and techniques that will render the extrapolations unnecessary is clear.

It is also easy to overlook the fact that the pressures in a centrifuge cell spinning at 1000 r.p.s. are not the atmospheric pressures at which ρ and v are actually measured. We were cautioned in this regard by the report of Trambarulo and Owen.[11]

With the aid of twin piezometers of 600 ml. capacity they have determined the compressibility of solutions of bovine serum albumin at pressures up to 500 bars and at protein concentrations up to 2.5%. The compressibilities of the solvent, a 0.1 normal sodium acetate buffer at the isoelectric pH, 4.73, of the albumin were also measured. Although the specific volume of the albumin decreases slightly with increasing pressure the predominant effect is that of the pressure at different levels in the centrifuge cell upon the density of the solution. For example, in a rotor spinning at 1000 r.p.s. where the pressure at the meniscus, $r_1 = 5.7$ cms., is 60 bars and that at the bottom, $r_2 = 7.3$ cms., is 470 bars, the solution at the bottom is some 0.016 g./ml. more dense than that at the top.[12] The corresponding variation in $(1 - \rho v)$ would be about 6%. In this extreme case, and in the absence of compensating effects from the pressure variation of other terms in equation 1, the sedimentation constant might be expected to decrease by this amount as the boundary moved through the cell. Much larger pressure effects should be observed with some of the commonly used non-aqueous solvents, and the importance of correcting for them is emphasized by the work of Mosimann and Signer.[13] However, at the relatively low speeds, 100 r.p.s. and less, and in the short cells that characterize the equilibrium centrifuge the pressure effects should be quite negligible, even in the case of most organic solvents.

Although not on the agenda it was inevitable that the question of protein

purity should arise at every session. There was general agreement that the homogeneity of the protein should be checked by all available means, i.e., sedimentation velocity, electrophoresis, solubility, crystallizability, etc. Moreover, repeated mention was made of the fact that many of the accepted methods for storing protein preparations, such as lyophilization, do not adequately preserve the material. McMeekin proposed a recrystallization of the preparation immediately prior to use and all confreres agreed that improved methods of storage should be sought. Experimental evidence, from sedimentation studies, of the difficulty of preserving the widely used bovine serum albumin that is available commercially was presented by Kegeles.

Sedimentation Equilibrium.—One of the most fruitful sessions of the conference was that in which the possibilities of the equilibrium ultracentrifuge were explored. The recent work of Williams and Wales[14] at Wisconsin on the sedimentation equilibria of both fractionated and unfractionated polystyrene has shown how the polydispersity of this material can thus be characterized if correction for the departure of the solutions from ideality is made with the aid of osmotic pressure measurements. Moreover, these investigators have solved, with the aid of the La Place transformation, the theoretical problem of the distribution function and now feel that the next most essential development is an increase in the precision with which the equilibrium concentrations can be determined.

In view of the persisting uncertainty as to whether the molecular weight obtained with the aid of the ultracentrifuge is that of the hydrated or anhydrous material, attention was called to the work of Pedersen[15] and Drucker[16] on salts. Since these workers obtained, after making the necessary activity corrections, a molecular weight corresponding to the formula weight the conclusion appears reasonable that the anhydrous weight is the quantity measured, since both salt and protein ions can be presumed to be hydrated. In the case, however, of the sedimentation velocity method some ambiguity still remains if the density of the "bound" water differs from that of the solution.

Since the equilibrium centrifuge generally spins at a lower speed than in the case of sedimentation many of the engineering problems are thereby reduced. Moreover, the following suggestion of Archibald[17] may eliminate the necessity of establishing equilibrium and thus reduce the time of observation.

In the approach toward equilibrium the flux, ϕ, of solute through unit cross-section of the cell is

$$\phi = \omega^2 rsc - D \frac{\partial c}{\partial r}$$

where the first term on the right provides for the transport of material by sedimentation and the second by diffusion. At equilibrium this flux is zero for all values of the radius, r. Since solute cannot move through the ends of the cell at r_1 and r_2, the flux is also zero at these two levels at all times, i.e., even prior to the attainment of equilibrium. Thus a plot of $1/rc$ $\partial c/\partial r$ against r should extrapolate to the same value, $\omega^2 s/D$, at r_1 and r_2 at any time, e.g., during the intermediate states that precede the establishment of sedimentation equilibrium. In contrast with the sedimentation velocity method the extrapolated intercept gives the value of the ratio, s/D, and hence for the computation of the molecular weight with the aid of equation 1 the only additional data required are the density, ρ, of the solution and the specific volume, v, of the protein. Unless a weight average value is adequate this method requires, as in the case of complete equilibrium, that the sedimenting material be monodisperse. Moreover, Pedersen noted that the extrapolation requires the greatest precision in the data that are most difficult to obtain, namely, the values near the meniscus and the bottom of the cell.

The foregoing procedure emphasizes the need for an optical method, such as the one described by Kegeles[18] utilizing a prismatic cell, that gives both c and $\partial c/\partial r$ as a function of r. The problem of cell shape remains, however, to be considered. In order to hasten the attainment of equilibrium it has become a general practice to take advantage of the convection that occurs in a channel of uniform cross-section. The theory provides, however, for transport by sedimentation and diffusion but not by convection. If the intermediate states are to be utilized as suggested by Archibald will it be necessary to use a sector-shaped cell as in sedimentation velocity measurements?

The adaptation of interferometry, using monochromatic light, to the equilibrium method involves optical problems peculiar to this procedure. In a sedimentation velocity experiment the solution above the boundary has essentially the same composition as that in the reference cell or channel. One thus has a known refractive index from which to count fringes. In the case of sedimentation equilibrium, however, at no level in the centrifuge cell is the refractive index identical with that in the reference cell. Even if the refractive index of the reference liquid were raised, by addition of a non-sedimenting solute, to a value corresponding to that at some level in the sedimentation cell this level could not be identified, except possibly by the use of white light. In view of the extreme precision that must be achieved in the determination of c and $\partial c/\partial r$ in the intermediate and equilibrium states it appears essential to continue the development of interference methods.

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NATIONAL ACADEMY OF SCIENCES
THE RANCHO SANTA FE CONFERENCE CONCERNING THE EVOLUTION OF THE EARTH

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At the meeting of the National Academy of Sciences in Rochester last October, Dr. Urey presented a paper entitled "On the Origin and Development of the Earth and Other Planets." His discussion was distinguished by its emphasis upon the many physical-chemical aspects of this broad subject. The initiation of the round-table discussion which I am privileged to report was a direct consequence of Dr. Urey's stimulating paper.

This conference, sponsored by the Academy, was held on January 23, 24, and 25 at Rancho Santa Fe, California. The discussion was informal, no prepared papers being presented. The subject, "The Evolution of the Earth," was discussed by a group of twenty-four scientists who represented the pertinent basic fields. Because of the recent stimulating contributions of chemists such as Urey, Latimer, and Harrison Brown, the group contained a high proportion of chemists; there were in fact seven chemists, six geophysicists, five geologists, and suitable representation in astronomy, fluid mechanics, physics, and oceanography.[1]

In every aspect of the discussions, the importance to the thinking of the group, of the contributions from all fields represented, was an outstanding feature. From the wealth of subject matter discussed I have selected a few topics for brief report. In this attempt to report upon the extensive informal discussions of the group, I must not imply that my selection of topics or the conclusions here inferred are necessarily representative of the opinions of the others in this group.

The conference opened with discussion of theories of origin of the solar system. In accordance with recent theories, the earth probably has grown by the accretion of relatively cool materials which were not molten at the outset. The chemists strongly favored the cool type of or.gin, for otherwise they are unable to account for the presence of water and the relative absence of the noble gases and the low abundance of nitrogen. Other chemical arguments were advanced in favor of the hypothesis of a relatively low temperature (about 300 or 400 degrees Kelvin) during the early growth of the earth. In later stages, some found need of temperatures high enough to produce general incipient melting of the crust, namely 1200–1500°K; but many of the geologists held that such temperatures on a world-wide scale, would have produced more complete layering of the crust and mantle than is observed. Both schools of thought of course agreed in requiring temperatures sufficiently high to produce at least

local melting of the crust or mantle. The question at issue was whether general melting of the crust on a world scale had ever occurred. On either hypothesis it still remains to understand how the iron (adopting the hypothesis of a molten iron core) got to the central core without leaving the mantle completely differentiated. Our conceptions of the development of the primitive earth are, to say the least, obscure. It is even uncertain whether the earth today is cooling or heating at depth, but the odds seem to favor the hypothesis of a heating earth.

The number of rock specimens whose age has been well measured, and the distribution of these specimens with respect to the important Pre-Cambrian structures on this continent, now seems to have arrived at an interesting stage. For the first time large-scale orderly patterns seem to be emerging which foretell the great potential fertility of the radioactive age measurement program. The status of the age determinations was reviewed, and measurements presented concerning eight major orogenic belts of the Pre-Cambrian. The successive positions and directions of these ong linear zones of activity were suggestive of successive stages in an orderly process of growth of the continent. The earliest dated orogeny, an east-west belt in Manitoba, Ontario and Quebec, is about 2.4 billion years old. Yet the geologic record shows that dry land existed in this area at even earlier times. The existence of dry land at the very beginning of the known geologic record is surely a significant point. The age measurements as a group tend to confirm the thesis of recurrent orogenic activity in the earliest geologic era of a type not basically different from that which is so well observed in recent geologic time.

It has generally been held that the *quantity* of radioactive heat production in the crust amply suffices to meet the energy requirements of the earth's internal geological machinery. This view was, I believe, the generally accepted view at the conference. However, the important question remains·· by what processes is this heat energy converted to the mechanical work of mountain building or continent building revealed in the geologic record?

The traditional basic mechanism for mountain building is the shrinking of a cooling earth. At our conference, however, many seemed to place faith in the alternative hypothesis of a heating earth; accordingly another method was needed for producing mountains and continents. The following process which seems to offer the ingredients for a suitable self-propagating mechanism was proposed and discussed. At a depth, the thick mantle of the earth probably has the approximate composition of stony meteorites or of peridotite. Bowen's classical studies at the Carnegie Geophysical Laboratory indicate that in a heating melt of peridotitic composition, the last crystals to melt are olivine and hypersthene, of high density. In a large magma chamber heating slowly, these will settle through the liquid

to form the ultra-basic rock known as dunite at the bottom of the chamber. The lighter fraction has a composition similar to that of the continental crust. The low melting components (alkalis) and radioactive elements are incorporated in this fraction; and this fraction can differentiate further depending on the history of cooling to form all the variety of igneous rocks which are known on the surface of the earth. A separation of radioactive materials by transport upward would produce smaller temperature grad ents and more uniform temperatures below.

The establishment of lower temperature gradients would increase the size of the temperature zone favorable for the incipient process of fractionation. Thus the differentiation would tend to encompass larger and larger volumes; it would in brief, tend to be self-propagating. A tendency for mountain building or continent building is obviously associated with such a process; namely, the tendency for the heavier dunite to sink and thereby initiate convective motion, and the tendency for the lighter fraction to rise.

The hypothesis of an essentially basaltic continent floating, so to speak, on a denser ultra-basic material is of course a very old one, but recent seismic evidence seems to furnish important new support to this concept. Under continents the major seismic discontinuity in the outer crust, known as the Mohorovičić discontinuity, usually occurs at depths of between 25 and 40 km. Beneath this discontinuity, occur rocks characterized by notably higher wave velocities, generally assumed to be ultra-basic. This discontinuity has been widely recognized under the continents, wherever seismic observations are available. However, Ewing's new seismic observations in the deep Atlantic basin, indicate the absence of this discontinuity and the absence of the thick basaltic layer under the Atlantic ocean. Instead, Ewing finds that the characteristic high velocity rocks occur close to the sea bottom, and thus at a much higher elevation. This configuration of the upper surface of the high velocity rocks, with a depression under the continents, is just about what is required to explain the isostatic balance between continents and ocean basins. The density difference between basalt and peridotite, and the thickness of the lighter continental layer of basalt are such as to satisfy Archimedes' requirements for hydrostatic equilibrium.

During the conference, important evidence was presented concerning the growth of the oceans and of the atmosphere throughout geologic time. But this material, so I understand, will be the subject of an early paper by Dr. Rubey; and I shall therefore not attempt to review this large subject now.

Such a conference seems to provide almost the ideal method for stimulating progress and for consolidating the thinking in so broad and complex a subject. The contributions from all the fields represented were almost

equally important and significant. In the words of one of our members "One of the striking results of the conference seemed to be the emergence of general agreement concerning the ideas presented, and the way these all point to a reasonable hypothesis for the growth of the continents." The values in a round table of this type probably cannot be fully recognized at once. But the ideas discussed, will, I believe, develop and mature over a period of years, stimulated at appropriate intervals, let us hope, by similar meetings from broad fields of science.

* The Institute's address is Los Angeles 24, California.

[1] Because of travel costs from the east a high percentage of this number came from California. Those attending the conference were: Norman L. Bowen, Harrison Brown, Perry Byerly, Carl Eckart, Maurice Ewing, James Gilluly, Edward Goldberg, David Griggs, B. Gutenberg, P. M. Hurley, J. G. Kirkwood, Adolph Knopf, W. M. Latimer, W. G. McMillan, Linus Pauling, Roger Revelle, H. P. Robertson, W. W. Rubey, L. B. Slichter, Edward Teller, Harold C. Urey, J. Verhoogen, Theodore von Kármán, F. L. Whipple, Oliver Wulf.

The National Academy of Sciences defrayed about 65 per cent of the cost of the conference and the Institute of Geophysics about 35 per cent.

In adjourning the conference, Dr. Pauling offered a motion requesting the chairman to circulate a list of names and addresses of those present in order that they might conveniently exchange reprints or other information, and possibly continue some of the unfinished discussions of the conference by correspondence.

THERMODYNAMIC ASPECTS OF A PROPOSED MECHANISM FOR ACETATE OXIDATION IN BACTERIA

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While thermodynamics does not pretend to postulate the mechanism for a given process, the details of which actually fall within the realm of kinetics, it does purport to predict the possible feasibility of such a mechanism. With a knowledge of the difference in energy and entropy between the starting substance and each of the intermediates involved, the question as to how far a given reaction may go can be answered with confidence without complicated experimentation. In this note the rationale of chemical thermodynamics has been applied to a mechanism recently proposed for the oxidation of acetate by *Escherichia coli*.

Ajl,[1] employing inhibition experiments with arsenious oxide and cyclohexanol as inhibiting agents and a modified version of the Stanier[2] technique of simultaneous adaptation, has obtained evidence against the occurrence of the Krebs oxidation cycle in the respiration of *E. coli* and *Aerobacter aerogenes*. From his findings he has been led to suggest the following series of steps in the oxidation of acetate by *E. coli*.


```
CH₃COOH   -2H   COOH   -2H   COOH  +HOH  COOH   -2H   COOH
    ⇌           |       ⇌     |      ⇌    |       ⇌    |      ⇌
    +           CH₂           CH          CHOH         CO
                |             ||          |            |
CH₃COOH   (1)   CH₂   (2)     CH    (3)   CH    (4)    CH    (5)
                |             ||          |            |
                COOH          COOH        COOH         COOH
Two moles of    Succinate     Fumarate    Malate       Oxalacetate
acetate condensing

                              COOH        + ½O₂        CH₃
                              |            ⇌           |
                              CO   + CO₂               |     + CO₂
                              |                        |
                              CH₃         (6)          COOH
                              Pyruvate                 Acetate
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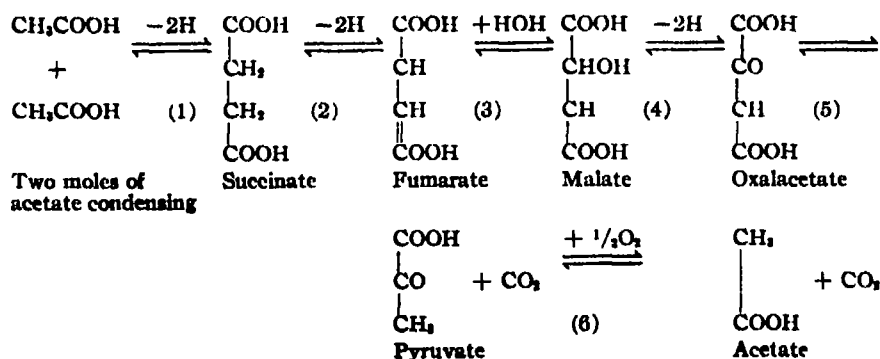
The free energy changes for each of these reactions can be calculated from the free energies involved in the formation of the respective compounds concerned, and these summed up to give the free energy change for the over-all reaction. Such calculations have been made using the values listed in table 1.

The free energy changes for the various steps of this system are given in table 2. Combination of the six steps gives an over-all balance of $-51,385$ cal., that is, the complete system is exergonic by a substantial margin.

TABLE 1

FREE ENERGIES OF FORMATION OF MOLECULES INVOLVED IN ACETATE OXIDATION BY *E. coli*

SUBSTANCE	$-\Delta F$,[a] CAL. MOLE $^{-1}$
O_2 (g, 0.2 atm.)	950
CO_2 (g, 0.0003 atm.)	99,060
H_2O (l)	56,560[3]
Ions	. . . . .
Acetate	89,700[4]
Succinate	166,360
Fumarate	142,525[5]
Malate	199,430[5]
Oxalacetate	184,210[5]
Pyruvate	106,460[5]

[a] Values calculated unless otherwise indicated.

TABLE 2

ACETATE OXIDATION BY *E. coli*

STEP	REACTION	ΔF, CAL.
(1) Condensation and dehydrogenation	Acetate + acetate $\rightleftharpoons$ succinate	+13,040
(2) Oxidation (dehydrogenation)	Succinate $\rightleftharpoons$ fumarate	+23,835
(3) Hydration	Fumarate + H_2O $\rightleftharpoons$ malate	−345
(4) Oxidation (dehydrogenation)	Malate $\rightleftharpoons$ oxalacetate	+15,220
(5) Decarboxylation	Oxalacetate $\rightleftharpoons$ pyruvate + CO_2	−21,310
(6) Oxidation	Pyruvate + $^1/_2O_2$ $\rightleftharpoons$ acetate + CO_2	−81,825
Over-all reaction postulated by Ajl	Acetate + H_2O + $^1/_2O_2$ $\rightleftharpoons$ $2CO_2$ + 6H	−51,385

By the transport of one pair of electrons from substrate to oxygen 3+ energy-rich phosphate bonds may be generated.[6] Energy-rich phosphate bonds average 12,000 cal. per bond. Hence, for the 3 pair of hydrogens evolved in the process and passing through the cytochrome system 108,000 cal. may be generated in the form of high phosphate bond energy. This

together with the 51,385 cal. resulting from the free energy changes in the over-all reaction gives a total energy of 159,385 cal. available for useful work.

The free energy change for the complete oxidation of acetate to carbon dioxide and water is −214,840 cal. However, Clifton and Logan[7] and Siegel and Clifton (experiments to be published) have demonstrated that *E. coli* oxidizes acetate to only three-fourths completion. These workers, employing manometric methods, found that with acetate as the sole substrate in a pH 7.2 phosphate buffer medium, washed cell suspensions carried out the oxidation to 74.9 per cent completion, and with a resultant R. Q. of 0.96. This suggested the stoichiometric equation for oxidative assimilation as being:

$$2C_2H_4O_2 + 3O_2 \rightleftharpoons (CH_2O) + 3CO_2 + 3H_2O.$$
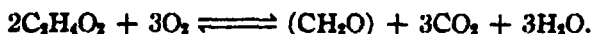
As a first approximation, the free energy change for the oxidative assimilation process was calculated to be −160,915 cal. (−214,840 × 0.749). This, according to thermodynamic reasoning, would be the energy actually available to the cell for the performance of useful work. As noted above, calculations made in line with the proposed mechanism for the acetate oxidation give a ΔF value of −159,385 cal. These two values are then in very close agreement, which might suggest that the process as proposed could be the correct one.

In any case, the mechanism for acetate oxidation by *E. coli* as intimated by Ajl's recent findings seems to have a thoroughly sound basis in thermodynamic formulations.

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[6] Lipmann, F., *Currents in Biochemical Research*, New York, 1946, pp. 137–148.

[7] Clifton, C. E., and Logan, W. A., *J. Bact.*, **37**, 523–540 (1939).

THE THICKNESSES OF HEMOGLOBIN AND BOVINE SERUM ALBUMIN MOLECULES AS UNIMOLECULAR LAYERS ADSORBED ONTO FILMS OF BARIUM STEARATE*

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The following work, which describes a method of measuring one dimension of some protein molecules, is based on the determination of the apparent thickness of a unimolecular layer of globular protein molecules adsorbed from solution onto a metallic slide covered with an optical gauge of barium stearate. Langmuir[1, 2] and Rothen[3] have published a few results obtained by such a technique, but have not exploited the method thoroughly. A complete set of experimental data has been obtained by Clowes[4] on insulin and protamine. He studied the effects of pH and time of exposure on the thickness of layers of protamine and insulin adsorbed onto slides covered with barium stearate and conditioned with uranyl acetate. He found that the pH was responsible for large variations in the thickness of the adsorbed layers and that the thickness of insulin layers adsorbed onto a protamine base was dependent on the concentration of the insulin. Since Clowes found thicknesses as high as 100 Å. for protamine and 400 Å. for insulin, he was without doubt usually dealing with multilayers.

Experimental.—The apparent thickness of a protein layer is measured with an optical instrument called the ellipsometer by Rothen,[5–7] who has given a complete description of its design and optics and has calculated its sensitivity as 0.3 Å. This instrument measures the ellipticity of light reflected from a metallic slide when it is covered with a thin film of transparent material. The parameters of the ellipse of polarization are determined in part by the thickness and refractive index of the transparent layer of material on the slide. The ellipsometer, which is a type of polarimeter, uses the half-shadow technique, by which a change in the ellipticity of the reflected light requires a change in angular setting of an analyzer used to balance the intensity of the half fields. As actually used, the instrument is calibrated with films of barium stearate, which for different known thicknesses, previously determined by x-ray diffraction measurements require different angular settings of the analyzer for equal intensity of the half fields. In this way one measures the angular change in the analyzer setting produced by a film of unknown thickness and relates it by a calibration curve to a known thickness of barium stearate.

Such a calibration assumes that the indices of refraction of films of

barium stearate and globular proteins are the same. The indices of refraction of hemoglobins and serum albumins in the unhydrated state have been calculated by Putzeys and Brosteaux[8] and by Armstrong[9] to be 1.60. Using data obtained by Bull[10] on the hydration of proteins in equilibrium with various partial pressures of water, one can correct the refractive index of 1.60 for hydration of the dry films resulting from the relative humidity of the laboratory. By using the Lorentz-Lorenz equation for such a mixture of protein and water, one obtains an effective refractive index of 1.57. However, it is reasonable to suppose that the globular protein molecules do not occupy the entire surface of the barium stearate, but pack together in such a way as to leave voids between themselves. This condition will further reduce the refractive index, which will be dependent on the type of packing assumed for the adsorbed protein molecules. It has been assumed that when the barium stearate surface is saturated with a monolayer of protein molecules the packing may be best approximated by the closest packing of elliptical cylinders resting on their bases. The fractional volume occupied by such a molecular model is 0.91, and if one assumes that the voids are occupied by air the effective refractive index, calculated by the Lorentz-Lorenz equation for a mixture, is 1.50. Since the refractive index of a barium stearate film is 1.50, the apparent thickness of a film of globular protein molecules, if one makes the above assumptions, is optically equivalent to the thickness of a film of barium stearate. Other assumptions concerning the degree of hydration and the percentage of voids would change the apparent measured thickness of the protein molecules by 1 or 2 Å.

The general technique used in these experiments was similar to that used by Rothen[3] in his work on antigen-antibody reactions. With the Blodgett and Langmuir[11] methods an optical gauge of barium stearate was placed on highly polished stainless steel slides which had been thoroughly cleaned with Shamva, a metallographic polish. The stearic acid, dissolved in redistilled benzene, was spread on redistilled water. The zero point reading was then determined on the ellipsometer. The carbonmonoxy-hemoglobin solutions were prepared from crystalline carbonmonoxyhemo-globin preparations obtained by the method of Drabkin,[12] and all dilutions were done with 0.003 M potassium phosphate buffer at pH 6.8. Concentrations of carbonmonoxyhemoglobin were determined colorimetrically on a Klett colorimeter, which had been calibrated by Kjeldahl analyses for nitrogen. The crystalline bovine serum albumin, obtained from Armour's Research Laboratories, was dissolved in acetate buffer of ionic strength 0.15 and pH 4.9.

For the adsorption it was found that the most reproducible results were obtained by placing the slides covered with barium stearate for five minutes in 5-ml. beakers containing the protein solutions. The slides were then

thoroughly washed in running distilled water for five minutes. For constant results a thorough and reproducible washing procedure was found to be of importance. After washing, the slides were allowed to dry in air, and the thicknesses of the adsorbed protein layers were then measured on the ellipsometer. All solutions and wash water were maintained between 16 and 19°C. The error for any one set of measurements was about 10%.

Results.—The results may be seen in Table 1 and Figure 1.

TABLE 1

THE APPARENT THICKNESSES OF SOME PROTEIN FILMS AT VARIOUS CONCENTRATIONS OF PROTEIN

CONCENTRATION, g./100 ml.	HUMAN CARBON-MONOXYHEMOGLOBIN, d in Å.	HORSE CARBON-MONOXYHEMOGLOBIN, d in Å.	BOVINE SERUM ALBUMIN, d in Å.
5	37	..	35
4	39	..	..
3	38	37	34
2	39	34	..
1	37	34	32
0.8	35	40	..
0.6	32	36	..
0.5	.	..	27
0.4	37	32	..
0.2	30	29	..
0.1	25	36	25
4×10^{-2}	19	..	..
2×10^{-2}	..	39	23
8×10^{-3}	12	..	..
1.6×10^{-3}	13	..	..
8×10^{-4}	..	33	..
2×10^{-4}	..	..	10
8×10^{-5}	..	35	..
5.3×10^{-5}	..	25	.
2×10^{-5}	..	..	0
5.3×10^{-5}	..	25	..

Interpretation of Results.—The data in Table 1 have been interpreted by using the simple Langmuir adsorption equation. If one assumes that the apparent thickness of the film, d, is proportional to the fraction of the surface covered, one obtains as the equation for the adsorption of a unimolecular layer of protein molecules onto a solid surface

$$C/d = 1/bd_m + C/d_m,$$

where

C = concentration of protein in g./100 ml.,
d = apparent thickness of the film,

d_m = the apparent thickness of a unimolecular layer of close-packed protein molecules, and

b = a constant related to the heat of adsorption.

It is seen that a plot of C/d against C should give a straight line, and that the reciprocal of the slope of this line is the apparent thickness of a unimolecular layer of protein molecules. Figure 1 shows that a straight line was obtained, and the reciprocals of the slopes of these lines give the thickness of the horse carbonmonoxyhemoglobin molecule as 36 Å., the human carbonmonoxyhemoglobin molecule as 38 Å., and the bovine serum albumin molecule as 34 Å.
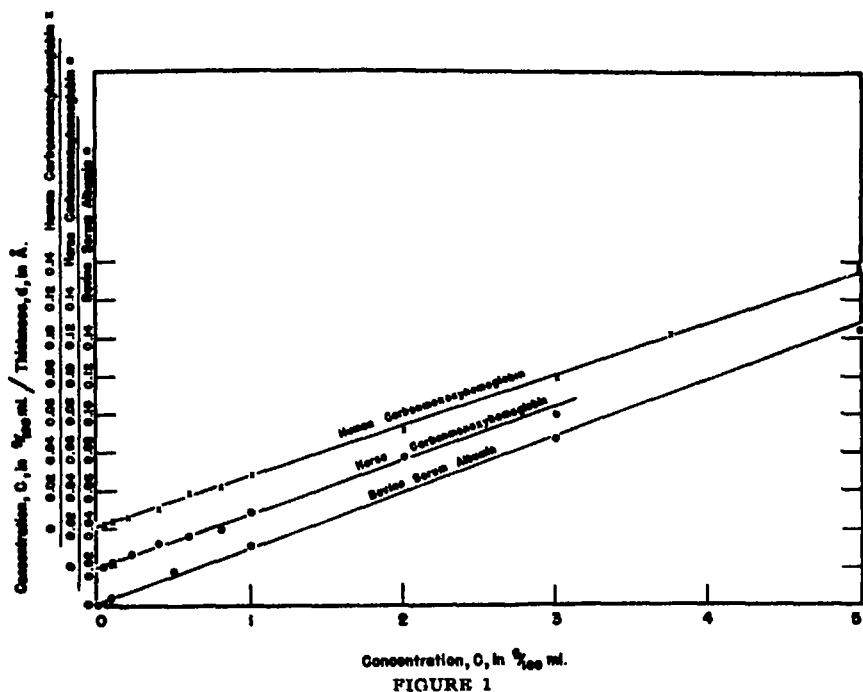
FIGURE 1

Langmuir adsorption isotherms for proteins adsorbed onto barium stearate, t = 16–19°C.

In one respect the conditions of our experiments departed from those assumed in the derivation of the adsorption isotherm equation; namely, that an equilibrium does exist between the molecules in solution and the adsorbed molecules. Our washing procedure amounted to placing the slide in an infinitely dilute solution, and one would therefore expect that, given enough time, all molecules would be desorbed. Our results indicate

that the five-minute period of washing was sufficient to remove any multi-layers of hemoglobin adsorbed onto the initial layer, but did not appreciably affect the number of molecules adsorbed onto the barium stearate. That the rate of desorption can be very slow is seen in the horse carbonmonoxyhemoglobin experiments, where concentrations of 8×10^{-6} g./100 ml. or 10^{-9} molar apparently still gave unimolecular layers.

A second factor to consider is the hypothesis that the apparent thickness of the film, d, is proportional to the fraction of the surface covered. The Lorentz-Lorenz equation for a mixture of protein and air may be written

$$(n^2 - 1)/(n^2 + 2) = fC_a,$$

where

n = refractive index of the mixture,
$C_a = (n_p{}^2 - 1)/(n_p{}^2 + 2)$,
n_p = refractive index of the protein, and
f = fraction of the surface covered.

Using the Drude equation, $\Delta = -A(1 - 1/n^2)d_m$, for the phase difference, Δ, between the components of the ellipse of polarization resulting from a film of actual thickness, d_m, and substituting for n, one obtains

$$\Delta = \frac{-3Ad_m}{2 + 1/(fC_a)},$$

where A is a constant. Since Rothen[7] has found that Δ is mainly responsible for the apparent thickness of the film, d, and a plot of the above equation in the region of physical significance shows that the relation between Δ and f is approximate proportionality, one is probably justified in using the Langmuir equation.

The value of 36 Å. for the thickness of the horse carbonmonoxyhemoglobin molecule may be compared with the value, 34 Å., obtained by Perutz[13] for the $c \sin \beta$ dimension in his x-ray diffraction work on horse ferrihemoglobin. It is believed that the close agreement between the two values, obtained independently, is significant.

The value of 34 Å. for the thickness of the bovine serum albumin molecule is to be compared with the value 40 Å. resulting from measurements on the double refraction of flow of bovine serum albumin made by Edsall and Foster[14] and with the value 38 Å. for the human serum albumin molecule obtained by Oncley, Scatchard and Brown.[15] Since the 40 Å. and 38 Å. values are for the minor axes of prolate ellipsoids of revolution, one would expect our value of 34 Å., which is an average thickness value, to be less.

The unimolecular layer method of determining one dimension of a globular protein molecule can presumably be applied to other proteins. In conjunction with the other methods of measurement it should prove

helpful in providing information about the size and shape of protein molecules.

The author is very grateful to Drs. Linus Pauling, Dan Campbell and John Singer for their interest and many helpful suggestions.

Summary.—By use of an optical method it has been found that the thickness of a unimolecular layer of human carbonmonoxyhemoglobin molecules adsorbed onto a film of barium stearate is 38 Å., that of horse carbonmonoxyhemoglobin molecules is 36 Å., and that of bovine serum albumin molecules is 34 Å.

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SEXUAL HORMONES IN ACHLYA. VI. THE HORMONES OF THE A-COMPLEX*

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The mechanism of hormonal control of sexual processes in heterothallic species of Achlya comprises a number of successive stages, each exhibiting complete dependence on that immediately preceding it.[1] Four morphological phases, each initiated and controlled by one or more specific hormones, are recognized: (1) the production of antheridial hyphae on the ♂ plant in response to the hormones of the *A-Complex* secreted by the vegetative ♂ and ♀ plants; (2) the production of oögonial initials on the ♀ plant in response to hormone B secreted by the antheridial hyphae; (3) the chemotropic attraction of antheridial hyphae to oögonial initials and the differentiation of antheridia in response to hormone C secreted by the oögonial initials; and (4) the delimitation of oögonia and the differentiation of the oögonial contents to form oöspheres in response to hormone D secreted by differentiated antheridia. The dual roles of hormones C and D indicate the probability that each is a hormonal complex consisting of two or more specific substances. Thus the interchange between the two sexes throughout the entire sexual reproductive process consists of specific, diffusible substances, with the exception of the physical transfer of ♂ nuclei in fertilization.

It was recognized early in the work that the ultimate understanding of the correlative mechanism must depend upon the stepwise elucidation of the successive stages, with concurrent development of the ability to control exactly those stages in the chain of events prior to that of immediate interest. A large part of the work has, therefore, been concentrated upon the initiation of the entire sexual progression (the production of antheridial hyphae) and the factors which quantitatively affect this response.[2,3] Continued work has furnished more exact detail concerning the activities of the hormones previously described and has revealed that two new hormones, one secreted by the vegetative ♀, the other by the vegetative ♂, are involved in the control of antheridial hyphal production. The activities of these two new hormones, particularly in combination with those previously described, necessitate a revision of the description of that portion of the hormonal mechanism pertaining to the production of antheridial hyphae.

The work reported here has been done with new isolates of ♂ and ♀ strains of *Achlya bisexualis* and *A. ambisexualis*.[4] All major results of the work prior to 1942 have been confirmed with these new isolates and it is thereby known that they are comparable with the isolates originally

employed. The testing methods for hormones affecting the production of antheridial hyphae have been described in detail elsewhere.[1, 2] The hormone concentrates which have been used include: hormone A "standard" solution in acetone containing 5×10^5 U./ml., prepared and standardized in 1948 from partially purified and highly concentrated material from filtrates of $A.$ *bisexualis* ♀ in 1943, and acetone-soluble and water-soluble (acetone-insoluble) fractions of filtrates from ♂ and ♀ strains of both heterothallic species. These latter fractions were prepared from each of the filtrates by the following procedure: diatomaceous earth, "Celite," was added to the filtrate and the water was removed from the slurry by distillation *in vacuo;* the dried material, deposited on the inert filler, was then exhaustively extracted with acetone in a Soxhlet's extraction apparatus to provide the acetone-soluble fraction; and the acetone-insoluble material on the filler was recovered by solution in water.

The initiation of the formation of antheridial hyphae on the ♂ plant occurs within an hour after the introduction of the test plants into water containing the appropriate hormones in the proper concentrations. By the end of three hours the antheridial hyphae are 100–500 μ in length and may be counted easily. Each vegetative hypha is treated as an individual and the average number of antheridial hyphae/3 mm. hyphal tip is taken as an index of the intensity of the reaction. Twenty to 100 or more individuals are counted for each test, the number depending upon the degree of accuracy required for the immediate purpose.

Four different hormones, collectively designated the *A-Complex*, are now known to affect quantitatively the production of antheridial hyphae. Two hormones, A and A^1, are secreted by the vegetative ♀ mycelium and each alone is capable of inducing the response. Two other hormones, A^1 (previously designated A')[2] and A^3, are secreted by the vegetative ♂ mycelium and neither can initiate the reaction. Since hormones A and A^2 are readily soluble in acetone or dioxane while hormones A^1 and A^3 are not, quantitative separation of the various hormones is readily accomplished.

The different hormones of the *A-Complex* will be considered in the order of their discovery.

Hormone A, secreted by the ♀, induces its characteristic response, the production of antheridial hyphae, with an intensity which is a logarithmic function of its concentration (the curve at the lower left of Fig. 1). This relationship obtains over a concentration range from 0.05 to 5000 U./ml., a factor of 100,000. Antheridial hyphae averaging fewer than 30/hyphal tip are most easily and accurately counted. Since maximal responses of this intensity are induced by approximately 50 U./ml., a greatly restricted range of concentrations, 0.5–50 U./ml., is therefore commonly employed.
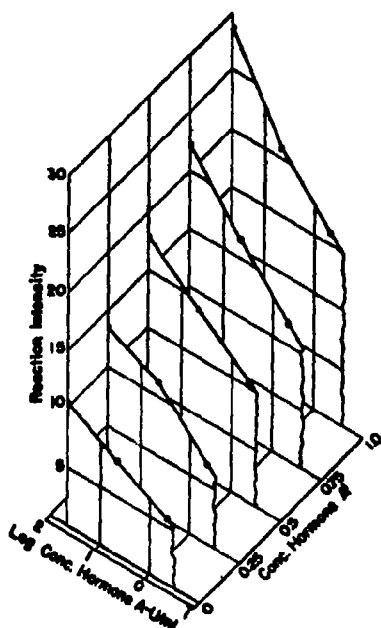
FIGURE 1
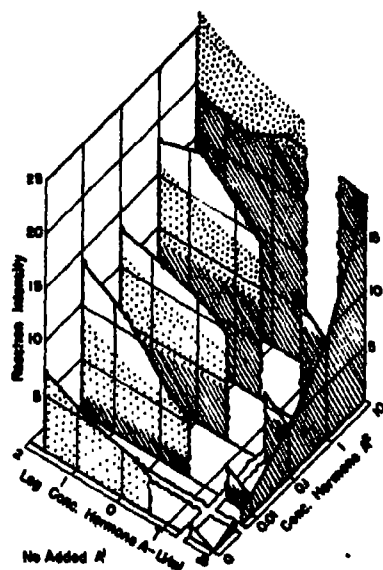
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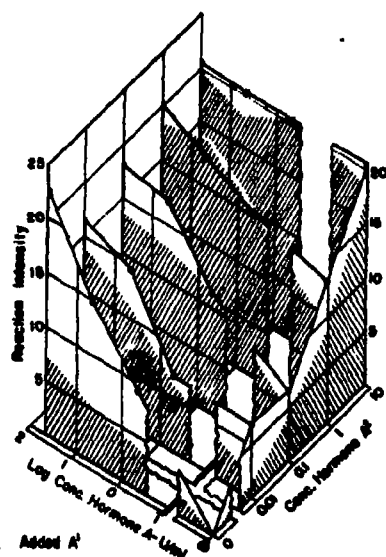
FIGURE 4
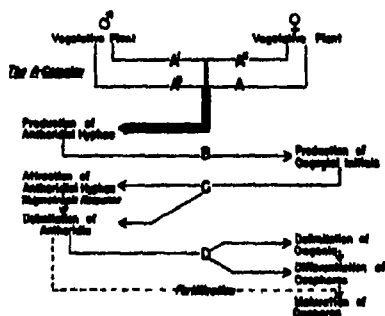
FIGURE 6

See opposite page for description of illustrations.

Hormone A^1, which is secreted by the vegetative $\male$ and has no initiating activity of its own, markedly enhances the intensity of the reaction to hormone A. The pattern of effect of A^1 on reaction intensity, at three concentrations of hormone A, is shown by the four upper curves of figure 1, representing four different concentrations of A^1. The responses elicited by hormone A in the absence of added A^1 is shown by the curve at the lower left of figure 1. The data plotted in figure 1 represent the average values for four tests, each point being the average reaction of 80 individual hyphae. From the data given in figure 1 and from the results of other series not included here, it is apparent that for a given concentration of hormone A an optimal concentration of hormone A^1 increases the intensity of the reaction by a factor of about three. For suboptimal concentrations of A^1, concentration of A remaining constant, the reaction intensity is roughly a linear function of A^1 concentration. The concentration range of A^1 over which this relationship holds, however, is very restricted as compared to that of hormone A; the greatest concentration of A^1 used here, that of raw filtrate from a mass culture of $\male$ mycelium, approaches an optimal value. Low concentration of either A or A^1 acts to limit the response. An intense production of antheridial hyphae is therefore possible only if both hormones are present in adequate amounts.

No method has yet been devised to determine whether A^1 is indispensable for the production of antheridial hyphae since the only means of testing for the hormone depends upon the reaction of the plant which simultaneously secretes it. Previous work has shown that amounts of A^1

Figure 1. Production of antheridial hyphae on the $\male$ plant in relation to graded concentrations of hormones A ($\female$) and A^1 ($\male$) when the two hormones are independently varied. In this and in subsequent figures the average number of antheridial hyphae/-3 mm. hyphal tip produced within four hours following the introduction of $\male$ plants into the test solutions is used as the index of reaction intensity and the concentrations of hormone A tested were 50, 5 and 0.5 U./ml.

Figure 3. Response, in the absence of added hormone A^1, to graded concentrations of hormone A alone (stippled curve at lower left) and hormone A^2 alone (lined curve at lower right) and to independently varied concentrations of both A and A^2 (four enclosed composite curves). The additive values for specific combinations of A and A^2 are indicated by the superposition of the curve for hormone A alone (stippled) on the projection of the control reaction for A^2 alone (lined) across each curve. Thus the unshaded portion of each curve represents the mutually augmentative effect of the two hormones in combination in excess of the sum of their separate activities.

Figure 4. The effect of added hormone A^1 on the responses induced by hormones A and A^2 in the same concentrations shown in figure 3. The response values for the various A plus A^2 combinations are shown by the shaded portions of each curve. Thus the unshaded portion, if any, of each curve represents the augmentative effect of hormone A^1 on the combined activity of hormone A plus A^2.

Figure 6. Revised diagram of the hormonal mechanism which initiates and co-ordinates the several phases of the sexual process in heterothallic species of Achlya.

significantly affecting the reaction are secreted by the ♂ plant during the 3–4-hour period required for the test.[2] The quantity of A^1 secreted into the test solution has also been shown to vary rhythmically, each cycle extending over 18–19 hours. Reasonably reproducible reactions may be obtained to a given concentration of hormone A over extended periods, however, if hormone A^1 is added in non-limiting quantity.

Hormone A^2, the third specific secretion involved in the control of the production of antheridial hyphae, is secreted simultaneously with hormone A by the vegetative ♀ and is chemically separable from hormone A. Hormone A^2, like A, induces the production of antheridial hyphae in the absence of other added hormones. The pattern of the response intensity
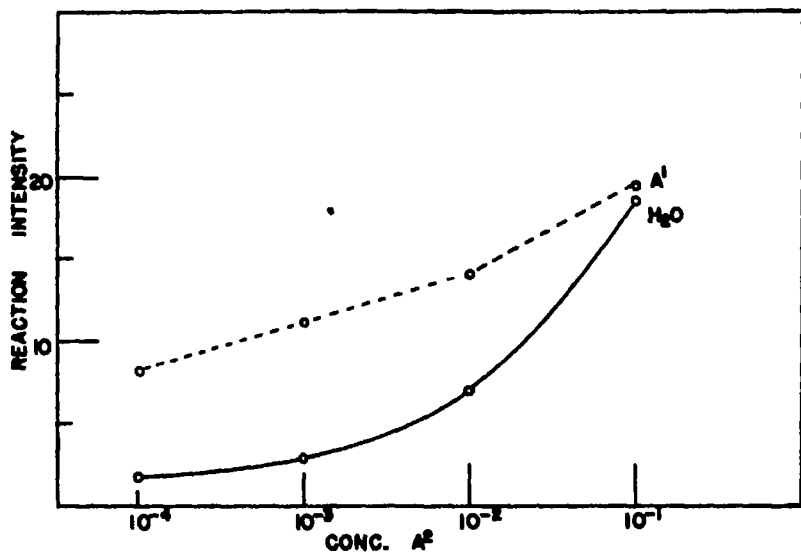
FIGURE 2

Response to graded concentrations of hormone A^2 (♀) in the absence (unbroken curve) and presence (broken curve) of added A^1.

vs. hormonal concentration is quite distinct from that of hormone A, the reaction intensity being roughly an exponential function[5] of the concentration of hormone A^2 (Fig. 2 and lower right curve of Fig. 3). The addition of hormone A^1 augments the response elicited by graded concentrations of hormone A^2 (Fig. 2). The augmentative effect of A^1 on A^2, however, does not resemble that on hormone A. Instead of increasing the response by a constant factor as with hormone A, its effect is most pronounced with low concentrations of A^2 and diminishes with increasing concentration of A^2, having but little effect when the latter is present at ten times its concentration in raw filtrate.

A still different pattern of augmentation results when hormones A and A^3 are combined.   A plot of reaction intensity as a function of concentration of hormones A and A^3, when the concentrations of the two hormones are independently varied, is given in figure 3.   Each of the two hormones is able, in the absence of the other, to induce the response in the intensities shown by the curves at the lower left and lower right of the figure.   The results which obtain in other portions of the plot, i.e., when both A and A^3 are simultaneously present, depend upon both the absolute quantities and the relative concentrations of the two hormones.   For example, when A^3 is present in a concentration of 0.01, the response intensity increases markedly with increase in the concentration of A, the reaction in each combination of A and A^3 being higher than the sum of the reactions separately induced by the two hormones.   The additive values for all combinations of A and A^3 are indicated in figure 3 by the superposition of the control response curve to hormone A alone (stippled) above the projection of the response to hormone A^3 alone (lined) across the face of each curve.   The greater-than-additive effectiveness of the A plus A^3 combinations (the unshaded portions of each curve) is thus seen to obtain in all cases except at the highest concentration of A^3.   Actually, the relationship shown in the three central curves of figure 3 probably obtains throughout the concentration ranges of both hormones found in matings of ♂ and ♀ plants.   The highest concentration of A^3 used here, 10 ×, would almost certainly never be encountered in a filtrate of ♀ plants or in the liquid in which plants are mated;   the concentrations of hormone A used here, however, fall within the range commonly present under mating conditions.

The response induced by combining hormones A and A^3 is further augmented by the addition of an optimal concentration of hormone A^1.   The pattern of this augmentation is shown in figure 4, in which reaction intensity (vertical coordinates) is plotted against independently varied concentrations of hormone A and A^3 in the presence of hormone A^1.   In this figure the reactions elicited by the specific combinations of A and A^3 in the absence of added A^1 (taken from the data presented in Fig. 3) are shown by the shaded areas on each curve.   Thus the augmentative effect of A^1 for the various combinations of A and A^3 is indicated by the unshaded areas on the several curves.   The augmentation by hormone A^1 of the response in the various combinations of the hormones A and A^3 is, however, less marked than its effect on either of the ♀-secreted hormones alone and it is greatly reduced in the presence of high concentrations of hormone A^3.   Actually, in certain combinations, the augmentative capacity of hormone A^1 would appear to be definitely decreased by hormone A^3, even in low concentrations of the latter.   Thus the response to 50 U./ml. of hormone A plus A^1 is reduced from an average number of 22.5 antheridial hyphae/ vegetative hypha to 16.1, 17.6, 20.1 and 20.4 by the addition of hormone

A^2 in concentrations of 0.01, 0.1, 1.0 and 10, respectively. Each of these points is based on the counts of 80–200 individual reacting hyphae and the decrease in each case due to hormone A^2 is unquestionably significant. This inhibitory effect is present, however, only at the single highest concentration of hormone A, 50 U./ml.[6]

Hormone A^3, the fourth secretion involved in the quantitative control of the production of antheridial hyphae, has been studied less intensively than the three hormones described above. This is especially true of the complex interactive effects in the various possible combinations with the other hormones. Hormone A^3 is secreted by the vegetative $\male$ plant simultaneously with hormone A^1, from which it is easily separable because of its ready solubility in acetone and dioxane, particularly the latter.
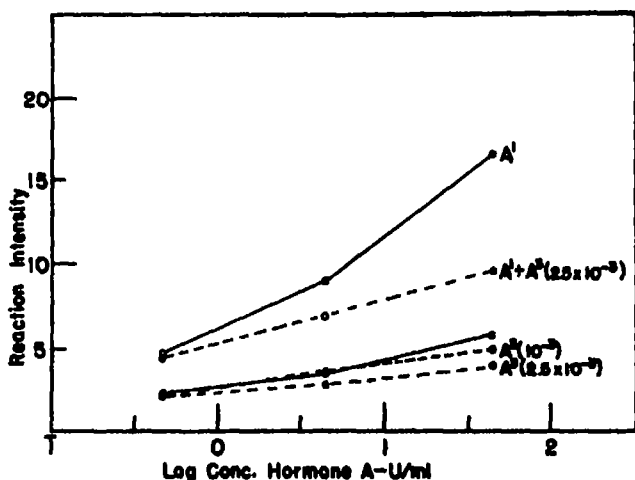
FIGURE 5

Suppression by hormone A^3 of the response induced by graded concentrations of hormone A in the presence and absence of hormone A^1. Solid curves represent control responses; broken curves, the suppressed responses induced in the presence of hormone A^3 (see text for concentrations).

Hormone A^3 acts to limit the number of antheridial hyphae produced by the plant in response to the other hormones of the A-Complex. This inhibition has been found to be effective on the response induced by both hormones A and A^2 and, most markedly, on that induced by hormone A in the presence of an optimal concentration of A^1. The pattern of inhibition by hormone A^3 of the response induced by hormone A in the presence and absence of added A^1 is shown by the broken curves of figure 5. The indicated concentrations of A^3 are those of an acetone solution of the hormone derived from the filtrate of mass culture of $\male$ mycelium; this solution contained hormone A^3 at 100 times its concentration in the raw

filtrate. The highest concentration used here, one-fourth that of the raw filtrate, significantly reduces the number of antheridial hyphae produced by A alone and by A plus A^1. The slight depression of the reaction by the lower concentration of A^3 at 50 U./ml. hormone A is of doubtful significance.

The effect of hormone A^3 on the reaction induced by A^2 has been tested only in a preliminary series. On the basis of these tests, a slight but significant decrease in the reaction induced by A^2 results from the addition of A^1. No attempts have been made to test the effect of A^3 in more complicated combinations of hormones A, A^1 and A^2.

The chemical identity of none of the hormones of the *A-Complex* is known; furthermore, but little is known about their chemical and physical properties. Certain of the properties of hormone A were described in connection with an unsuccessful attempt to isolate and identify the hormone,[3] but insufficient information was obtained even to assign the category of organic compounds to which the hormone belongs. Hormone A^1 has been found, in preliminary studies, to be readily dialyzable, neutral in reaction, non-migratory in an electric field, quite stable to heat, acid and oxidizing agents, and destroyed by prolonged alkaline hydrolysis; it is readily soluble in water but no other adequate solvent for it has been found. Nothing whatever is known of hormones A^2 and A^3 beyond their solubilities in water and acetone (or dioxane), respectively. Chemical characterization and identification of these hormones of the *A-Complex*, as well as the other hormones effective in the sexual process of Achlya, have been and will continue to be seriously hampered by the relative difficulty of obtaining the raw materials in sufficient quantities.

The four hormones of the *A-Complex* are secreted by vegetative plants in the presence or absence of sexually compatible strains. Furthermore, sufficient interspecific testing has been done to show that the secretions of ♂ and ♀ strains of at least two heterothallic species, *A. bisexualis* and *A. ambisexualis*, are completely comparable and that the A-hormones of either species are completely effective on the ♂ strain of the other species. It does not necessarily follow from this fact that the hormones of the *A-Complex* secreted by the two species are chemically identical.

The titers of three of the four hormones of the *A-Complex* are known to vary characteristically with the age of the cultures in which they are produced. The titer of hormone A, in ♀ cultures, remains low for the first week of vegetative growth after which it increases rapidly to reach a maximum at 10–12 days. Hormone A^1, in ♂ culture, reaches a maximal concentration three to five days after the initiation of vegetative growth and thereafter steadily decreases to approximately one-half its maximal activity at 14 days. The titer of hormone A^3, also in ♂ cultures, builds up to a maximum within the first five days of growth and remains constant

thereafter. No specific study has been made of activity of hormone A^2 (♀) in respect to culture age.

A revised scheme for the mechanism of hormonal control in heterothallic Achlyas, including the two new members of the *A-Complex*, hormones A^2 and A^3, described here for the first time, is given in figure 6. Only the first of the several stages in the sexual reaction, that pertaining to the production of antheridial hyphae, has been subjected to intense quantitative study and it is not unlikely that the hormonal mechanism, as presented here, will require many further revisions as subsequent stages are successively brought under intensive examination.

Summary.—Two new hormones, A^2 and A^3, secreted by vegetative mycelia of ♀ and ♂ strains, respectively, have been shown to be involved in the initiation of the sexual reaction in heterothallic species of Achlya. Thus the quantitative control of the production of antheridial hyphae on ♂ plants depends upon four distinct hormones: hormones A and A^2, secreted by the ♀, and hormones A^1 (originally designated A') and A^3, secreted by the ♂. These hormones regulate the production of antheridial hyphae in such a way that the intensity of the reaction is: (1) a logarithmic function of the concentration of A, (2) an exponential function[5] of the concentration of A^2, (3) a linear function of the concentration of A^1 in the presence of A, (4) increased by A^1 in the presence of A^3 (the precise pattern of augmentation not yet determined), (5) roughly a logarithmic function of the concentration of A^2 in the presence of A or in the combined presence of A plus A^1, and (6) decreased by A^3 in the presence of A or A^2 alone and in the combined presence of A plus A^1. A revised diagram of the entire hormonal mechanism, including the above additions to the *A-Complex*, is given.

[1] Raper, John R., *Science*, 89, 321 (1939); *Am. J. Bot.*, 26, 639 (1939); *Ibid.*, 27, 162 (1940).

[2] Raper, John R., *Am. J. Bot.*, 29, 159 (1942); Proc. Natl. Acad. Sci., 28, 509 (1942).

[3] Raper, John R., and Haagan-Smit, A. J., *J. Biol. Chem.*, 143, 311 (1942).

[4] Collection data, etc., of these materials are given in: Raper, John R., *Bot. Gaz.* (in press). Cultures of ♂ and ♀ strains of *Achlya bisexualis* and *A. ambisexualis*, as well as a number of sexual intergrade strains of the latter species, have been deposited in the Centraalbureau voor Schimmelcultures, Baarn, Holland.

⁸ A plot of log log antheridial hyphae vs. log concentration of A^3 reveals a linear relationship. From this it follows that $I = e^{ax^2}$, where I is reaction intensity, x, the concentration of A^3 and k, a proportionality constant. The author wishes to thank Dr. Leonard J. Savage for pointing out this relationship.

⁶ An alternate interpretation of A^3 activity might be that the water-soluble fraction of ♀ filtrate contains A^1 plus an effective contamination of A. The pattern of A^1 augmentation on A^3 response and the expected response curve of an $A + A^1$ mixture, when the two hormones are simultaneously diluted, lend strong support for such an hypothesis. On quantitative grounds, however, it is apparent that no possible mixture of hormones A and A^1 could give either the augmentative or inhibitory effects attributed to hormone A^3 when the water-soluble fraction is added to known concentrations of hormone A in the absence and presence of A^1, respectively (Figs. 3 and 4). It is because of these latter effects that the interpretation presented in this paper is favored over that of a mixture of previously known hormones.

ELECTRON TRANSFER IN INTERMETALLIC COMPOUNDS

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In our discussions of the electronic structure of intermetallic compounds during the last three years brief mention has been made from time to time of the phenomenon of electron transfer.[1-3] The interpretation of the observed interatomic distances in many metallic phases seems to require the assumption that electron transfer has taken place; an example is Al_9Co_2, for which the distances[4] support the charge distribution[5] $Al_9^{-2}/^9Co_2^{+1}$. The indication by physical properties of a filled-Brillouin-zone structure for Fe_5Zn_{21} also led to the suggestion of electron transfer:[2] about $^1/_4$ electron is indicated to have been removed from each zinc atom and about one electron added to each iron atom.

In this paper it is pointed out that the analysis of interatomic distances shows that electron transfer takes place in a great many interatomic compounds, and that the numbers of electrons involved are reasonable, in relation to the changes in valence resulting from loss or gain of electrons and to the partial ionic character of the bonds between unlike atoms and the striving of atoms toward electroneutrality.

Let us divide atoms into three classes: hypoelectronic (electron-deficient) atoms, hyperelectronic (electron-excess) atoms, and buffer atoms. Hypoelectronic atoms are atoms that can increase their valence by adding electrons. The hypoelectronic elements include the first three elements of each short period and the first five elements of each long period, as shown in

table 1. Atoms of these elements have more bond orbitals than valence electrons (in the uncharged state), and they can accordingly increase their valence by one unit by accepting an electron. Hyperelectronic atoms are atoms that can increase their valence by giving up an electron. The hyperelectronic elements with respect to metallic compounds include the last three elements (before the noble gases) of each short period and the last seven elements of each long period. Atoms of these elements have more valence electrons than bond orbitals, and they can increase their valence by one unit by giving up one electron of a pair occupying a bond orbital, thus leaving a valence electron in the orbital. Buffer atoms are atoms that can give up or accept an electron without change in valence. The five elements Cr, Mn, Fe, Co, and Ni and their congeners in the other two long periods are buffer elements with respect to metallic compounds;

TABLE 1

CLASSIFICATION OF ATOMS WITH RESPECT TO EFFECT OF CHANGE OF ELECTRON NUMBER ON METALLIC VALENCE

HYPOELECTRONIC ATOMS					ATOMS WITH STABLE VALENCE					HYPERELECTRONIC ATOMS					
Li	Be	B			C					N	O	F			
Na	Mg	Al			Si					P	S	Cl			
					BUFFER ATOMS										
K	Ca	Sc	Ti	V	Cr*	Mn	Fe	Co	Ni	Cu	Zn	Ga	Ge	As	Se	Br
Rb	Sr	Y	Zr	Nb	Mo*	Tc	Ru	Rh	Pd	Ag	Cd	In	Sn	Sb	Te	I
Cs	Ba	La	Ceᵇ												
		Lu	Hf	Ta	W*	Re	Os	Ir	Pt	Au	Hg	Tl	Pb	Bi	Po	At

* These three atoms can accept electrons but not give up electrons without change in valence.
ᵇ The rare-earth metals may have some buffering power.

they can give up a non-bonding d electron or introduce an electron into the incomplete non-bonding d subshell without change in metallic valence (Cr, Mo, and W are buffer atoms with respect only to addition of an electron).

Carbon and silicon are placed in a separate class in table 1. Carbon is an element with stable valence, 4; either the addition of an electron to a carbon atom or the removal of an electron from it causes a decrease in its valence. Silicon also has the stable valence 4, except that it may under certain circumstances make use of outer orbitals ($3d$, $4s$, $4p$) and achieve some increase in valence through electron transfer. This effect is less important in alloys of silicon than in compounds of the hypoelectronic atoms.

Let us consider the ways in which an intermetallic compound AB might be stabilized by the transfer of an electron from atom B to atom A.

First, an increase in the number of valence bonds and a corresponding increase in stability would result from electron transfer from B to A if A

were hypoelectronic and B were hyperelectronic, or if A were hypoelectronic and B were a buffer, or if A were a buffer and B were hyperelectronic.

Second, according to the principle of electroneutrality[4] an increase in stability would result from a transfer of electrons if it were to result in a decrease in the electric charges on the atoms. Let B be more electronegative than A. The covalent bonds between A and B would then have some ionic character, of such a nature as to give A a positive electric charge and B a negative charge. By transferring an electron from B to A the charges on the atoms can be reduced, and the substance can thus be stabilized. It is very interesting that this effect involves the transfer of electrons to the more electropositive atoms (the stronger metals); that is, in the opposite direction to that of the transfer of electrons that takes place in the formation of ions in electrolytic solutions.

These two stabilizing effects usually operate together, because the electronegativity increases in the sequence hypoelectronic elements, buffers, hyperelectronic elements. Both effects are stronger for compounds of hypoelectronic elements with hyperelectronic elements than for compounds of elements of either of these two classes with buffer elements. Thus we expect electron transfer to be especially important for compounds such as $NaZn_{13}$, less important for compounds such as Al_9Co_2 and Fe_5Zn_{21}, and of little significance for compounds such as Na_2K, FeCr, and Cu_5Zn_8.

In special cases electron transfer may take place even in compounds of two metals in the same class. Stabilizing factors that might operate to this end include the filling of Brillouin zones, the stabilizing of partially filled non-bonding subshells through increase in multiplicity (approach to half-filling) or through completion of the subshell, and the relief of strain resulting from geometric constraints on ratios of interatomic distances through change in bond numbers.

The compound AlP may be taken as a simple example. It has the sphalerite structure, in which each atom is surrounded tetrahedrally by four unlike atoms. Aluminum is a hypoelectronic atom, with normal valence 3 and with single-bond radius 1.248 Å. Phosphorus is a hyperelectronic atom, with normal valence 3 (resulting from occupancy of four orbitals by five electrons) and single-bond radius 1.10 Å. The predicted Al—P distance for valence 3 and bond number n equal to $^1/_4$ is $1.25 + 1.10 - 0.600 \log n = 2.43$ Å. The observed distance 2.35 Å., does not agree with this value, but is exactly equal to the sum of the single-bond radii; that is, the observed distance indicates that each atom forms four single bonds with its neighbors. We are thus led to describe the crystal by saying that it is composed of quadrivalent·$\dot{\text{A}}\text{l}^{-}$· and ·$\dot{\text{P}}^{+}$·, with formal charges -1 and $+1$, respectively; and that, because phosphorus is more electronegative than aluminum, each of the four single bonds formed by

each atom has about 25% ionic character, in such direction as to restore the transferred electrons from the aluminum atoms to the phosphorus atoms, achieving at the same time the extra stability that results from ionic-covalent bond resonance.

Comparison of observed and predicted interatomic distances for the thirty other tetrahedral compounds of this type shows that in general electron transfer occurs, with increase in valence of about one unit for each atom.

As another example we may discuss the striking purple alloy Al_2Au, which has the fluorite structure, with $a_0 = 5.90$ Å. Each gold atom has eight aluminum ligates, at 2.59 Å. If gold retained its usual metallic valence, $5^1/_2$, the eight Au—Al bonds would have bond number 11/16, and the corresponding correction $-0.600 \log n = 0.098$, plus the single-bond radii 1.338 for gold and 1.248 for aluminum, would give the predicted Au—Al distance 2.684 Å., which is so much greater than the observed value as to eliminate the assumed valences. Agreement is obtained by assuming gold to have the valence 7; the corresponding radius[8] is 1.303 Å., and the bond-number correction, for $n = 7/8$, is 0.035, leading to 2.586 Å. for Au—Al.

The valence 7 can be achieved by a neutral gold atom (without a metallic orbital). However, in order for gold to have valence 7, aluminum must have valence $3^1/_2$, or greater if significant Al—Al bonds are formed; and hence at least one electron per gold atom must have been transferred to the aluminum atoms. Indeed, the observed Al—Al distance 3.00 Å. for the six aluminum ligates about each aluminum atom corresponds to $n = 0.15$, and indicates that a significant amount of valence of the aluminum atoms is used in these bonds. It is likely that about 1.5 electrons are removed from each gold atom, which would liberate the customary 0.75 metallic orbital, the valence remaining 7; 0.75 electron added to each aluminum atom would increase the aluminum valence to 3.75, of which $3^1/_2$ would be used in bonds to the four gold ligates, and the remainder in Al—Al bonds.

This large amount of electron transfer is not incompatible with the electroneutrality principle. The electronegativity of aluminum is 1.5, and that of gold is 2.5. The difference corresponds to 22% ionic character of the Au—Al bonds, which with valence 7 for gold would lead to the charge -1.54 on the gold atom. To restore it to neutrality 1.54 electrons would have to be transferred to two aluminum atoms.

The proposed structure provides an explanation of the very high melting point (1060°C.) and large heat of formation of the compound.[7] Coffinberry and Hultgren[8] pointed out that the properties of the Al—Au alloys indicate the operation of an unusually strong attraction between aluminum atoms and gold atoms.

As an example of a compound in which electron transfer is relatively unimportant we may discuss PtSn$_2$, which also has the fluorite structure, a_0 being 6.41 Å. The normal metallic valences 6 for platinum and 4 for tin permit the formation of Pt—Sn bonds with $n = {}^2/_3$ and Sn—Sn bonds with $n = {}^1/_6$. The predicted Pt—Sn distance 1.295 + 1.399 + 0.106 = 2.800 Å. is only slightly high, the observed distance being 2.78 Å. The predicted Sn—Sn distance for $n = 1/6$, 3.27 Å., is also slightly higher than the observed value, 3.21 Å., and a small amount of electron transfer is accordingly indicated. The electronegativity values, 2.2 for platinum and 1.7 for tin, lead to about 6% ionic character of the bonds, and to charges on the atoms that would be neutralized by the transfer of 0.36 electron from each platinum atom to two tin atoms. The increase in valence of tin by 0.18 would increase n for the Sn—Sn bonds to 0.20, and would decrease the radius of tin[3] by 0.01 Å.; the predicted distances Pt—Al = 2.79 Å. and Al—Al = 3.20 Å. are in excellent agreement with the observed values.

Electron transfer is especially important in the alloys of the alkali and alkaline-earth metals with hyperelectronic elements and buffer elements. In the formation of many of these alloys from the elements a very large volume contraction is observed, resulting in part from the bond-number correction of interatomic distances due to the increase in valence, and in part from the decrease in single-bond radius of the hypoelectronic atom with increase in valence. Thus, although the normal radius of sodium for ligancy 12, 1.896 Å., is greater than that of lead, 1.746 Å., the replacement of one fourth of the lead atoms in pure lead by sodium atoms, to form the phase NaPb$_3$, leads to a contraction, the bond distance decreasing from 3.492 Å. to 3.446 Å. This decrease is explained by the assumption that electron transfer occurs, with a little less than one electron transferred to the sodium atom. For Na$^-$, with valence 2, the single-bond radius would be predicted to be 1.439 Å. (0.075 Å. greater than for Mg), which leads to Na—Pb = 3.413 Å., slightly less than the observed value, and the Pb—Pb distance is also predicted to be decreased by about the observed amount, as the result of the increase in valence of lead and in bond number of the Pb—Pb bonds. In many other intermetallic compounds of the alkali and alkaline-earth metals the interatomic distances similarly indicate that electron transfer occurs to such an extent as to increase the valence by about one unit.

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IMMUNOGENETIC AND BIOCHEMICAL STUDIES OF NEUROSPORA CRASSA: DIFFERENCES IN TYROSINASE ACTIVITY BETWEEN MATING TYPES OF STRAIN 15300 (ALBINO-2)*

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The concept that primary gene action consists of the determination of protein specificities and that genes bear a one-to-one relation to such specificities rests largely on biochemical investigations utilizing Neurospora and immunogenetic investigations of vertebrate erythrocytic antigens.[1,2] The fruitfulness of this hypothesis has been large, but questions have been raised as to the suitability of the methods and materials used in the above investigations.[3,4] Accordingly we have recently initiated a series of studies utilizing a new approach.

The material chosen for this work has been the two mating types (*A* and *a*) of Strain 15300 (albino-2), *Neurospora crassa*.[5] Mating type, which may be regarded as a specificity difference rather than a true sexual difference, is determined by two alleles (*A* and *a*) at a locus on the opposite side of the centromere of chromosome I (linkage group *A*) from the locus of albino-2.[6] The object of these studies has been to demonstrate possible differences in antigenic specificity and enzymatic specificity attributable to the mating type locus in addition to the mating type difference.

The results of the antigenic analysis indicate clear-cut antigenic differences between the two mating types.[7] In the course of this work it was necessary to prepare homogenates of whole mycelia. These homogenates were prepared from mycelia of strains 15300*a* and 15300*A* cultured, without shaking, in 100-ml. portions of medium in 250-ml. Erlenmeyer flasks. The medium employed consisted of 5 g. of KH_2PO_4, 7 g. of Difco Yeast Extract and 50 g. of dextrose which were dissolved in distilled water and the volume made to one liter. The sterile medium was inoculated and the cultures were incubated at 30°C. for twelve or thirteen days, after which time the thick mycelial pads were removed by filtration, washed

thoroughly with tap water and then thoroughly with distilled water. The pads were then squeezed to remove as much water as possible, cut into small squares, and lyophilized to complete dryness. The dried mycelium was then reduced in a Waring blendor in 0.85% NaCl buffered at pH 7.4 with 0.005 M phosphate (1 g. dried mycelium per 100 ml. saline), and the resultant suspension homogenized in a Potter-Elvehjem homogenizer. The homogenate was dispensed into small tubes, quickly frozen and stored in a deep-freeze until used.

' Shortly after the antigenic analysis was initiated it became apparent that there existed a hitherto unreported difference between these two mating types. First, it was noted that when homogenates prepared as described above were allowed to thaw, one darkened rather rapidly at room temperature while the other did not change color. Second, the mycelium (growing on agar slants) of one mating type was observed to darken with age whereas the other did not. In both instances the darkening was due to the production of a brown or black pigment and occurred only in homogenates or old cultures of 15300a. While slight darkening of old cultures of 15300 had previously been reported,[1] no difference between the two mating types had been noted. That this difference is not characteristic of our stocks alone was vouchsafed by a similar difference between new cultures of 15300a and A obtained from Dr. G. W. Beadle. The present paper is a report on the preliminary investigations concerning the nature of this difference.

Throughout this work homogenates were prepared as described above. At the time of use they were allowed to thaw at room temperature, were centrifuged at 6°C. (5000 r. p. m., 25 minutes), and the clear supernatant liquid was pipetted off and used immediately. Optical densities of these supernates were measured by means of a Klett-Summerson photoelectric colorimeter, the blue filter (No. 44) being used for such measurements. During the course of an experiment the supernates, or the mixtures of supernates and test substrates, were incubated at 37.5°C. Test substrates were dissolved in the buffered saline described above. Unless otherwise indicated, the tyrosine solutions used were 0.04 per cent; dopa solutions were made to a concentration of 1 mg./ml. Fresh solutions were made immediately preceding each experiment.

Since the characteristic color of a darkened homogenate was brown or black, it was early suspected that the darkening was due to melanin and that (1) one strain produced a phenol oxidase while the other did not, or (2) both strains produced phenol oxidase but only one (the strain which darkened) synthesized substrate which could be oxidized with resultant melanin formation. In an early experiment pigment development was followed by measuring optical densities (D) of supernates of both mating types at intervals, and it was found that the optical density of the supernate

of 15300a increased for about forty minutes and then came to a constant value; a parallel tube of 15300A supernate showed no increase in optical activity (Fig. 1). Since phenol oxidase activity was suspected, a 0.04 per cent tyrosine solution was added (1:1) to each of the two supernates
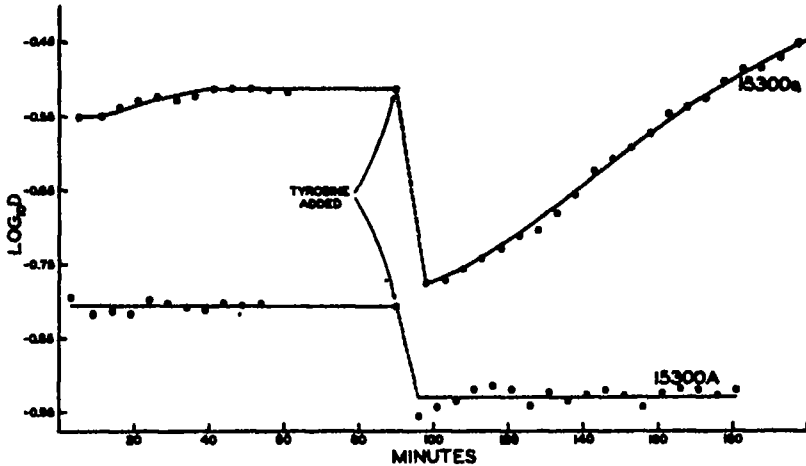
FIGURE 1

Pigment development exhibited by 15,300A and 15,300a extracts prior to and following addition of tyrosine.
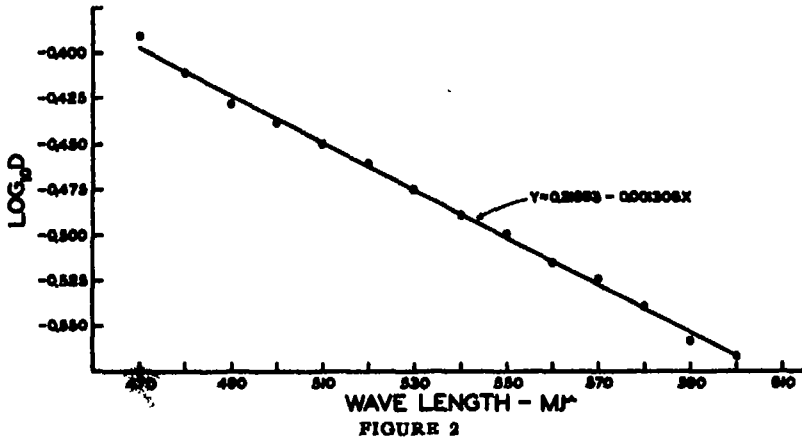
FIGURE 2

Absorption spectrum of pigment developed by 15,300a extracts.

and optical density measurements were continued. With 15300A there was no increase in optical density, while with 15300a there was an immediate rise, the magnitude of which is shown in figure 1.

In order to make a more positive identification of the pigment, a tube

of 15300ₐ supernate, to which tyrosine solution had been added, was allowed to incubate until a deep color was developed. Percentage absorption of light of different wave-lengths by this solution was then measured with a Beckman quartz spectrophotometer and an absorption curve
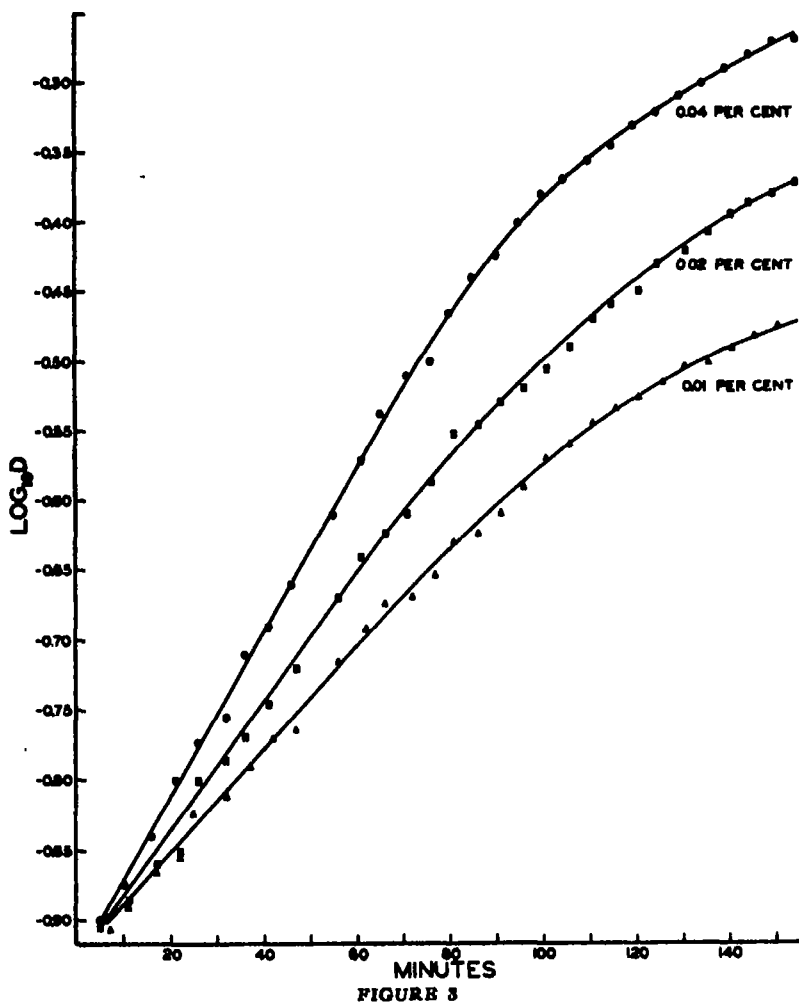
FIGURE 3

Effect of substrate concentration (tyrosine) on activity of 15,300ₐ extracts.

was plotted. This curve, part of which is presented in figure 2, is a straight line between 470 and 600 mμ—a characteristic of the absorption curve of melanin as reported by other investigators.[4, 9, 10] The equation for the straight line fitted to this portion of the curve by the least squares method is $Y = 0.21693 - 0.001306X$, the slope of the curve being closely similar

to that previously reported for melanin. The pigment is furthermore decolorized by potassium permanganate, as is melanin, so that there is little doubt about its identity.

That an enzyme is involved in melanin formation in the present instance is evidenced by the following: (1) no increase in optical density occurred when a boiled supernate was used, (2) after the optical density of 15300a supernate reached a constant value there was a resumption of pigment formation with addition of proper substrate, (3) there was a decline in activity of thawed homogenates with age, and (4) activity of 15300a supernates proved sensitive to pH and electrolyte concentration.

Further evidence that an enzyme is involved in melanin formation was supplied by the results of an experiment in which a supernate of 15300a was allowed to develop maximum color and 5-ml. portions were then added to equal volumes of tyrosine solutions of different concentration (0.01, 0.02 and 0.04 per cent). The results of this experiment, presented graphically in figure 3, show that which is true for most enzyme reactions, i.e., the velocity of the reaction increases with increase in substrate concentration, enzyme concentration being the same.

In another series the tyrosinase activities of 1:2, 1:4 and 1:8 dilutions of supernate of 15300a were tested, and the reaction velocities were found to decrease with increased dilution of the enzyme extract (Fig. 4).

Some investigators are of the opinion that mono- and polyphenol oxidases are in reality the same enzyme; therefore, an enzyme capable of oxidizing a monophenol compound such as tyrosine should also have capacity to oxidize a polyphenol compound.[11] In order to determine if this is true for the tyrosinase of 15300a, a series of experiments was conducted to determine if this enzyme would also oxidize dopa (1-dihydroxyphenylalanine). Since dopa undergoes rapid autoxidation, it was necessary to prepare three separate systems for each determination: (1) dopa + supernate, (2) buffer + supernate and (3) dopa + buffer. These mixtures were incubated for two hours, and from the optical density measurements made on the three separate systems, activity indices were calculated according to the equation used by Ginsburg:[10]

$$A = \frac{D_r - D_m}{D_d},$$

where

D_r = optical density—extract + dopa,
D_m = optical density—extract + buffer,
D_d = optical density—buffer + dopa.

An activity index greater than 1 indicates enzymic oxidation of the substrate, an activity index of 1 indicates no enzyme-catalyzed oxidation,

and an index of less than 1 is indicative of inhibition of substrate oxidation. Results of five experiments are presented in table 1,. demonstrating quite clearly the presence of dopa oxidase in 15300a but not in 15300A.   The
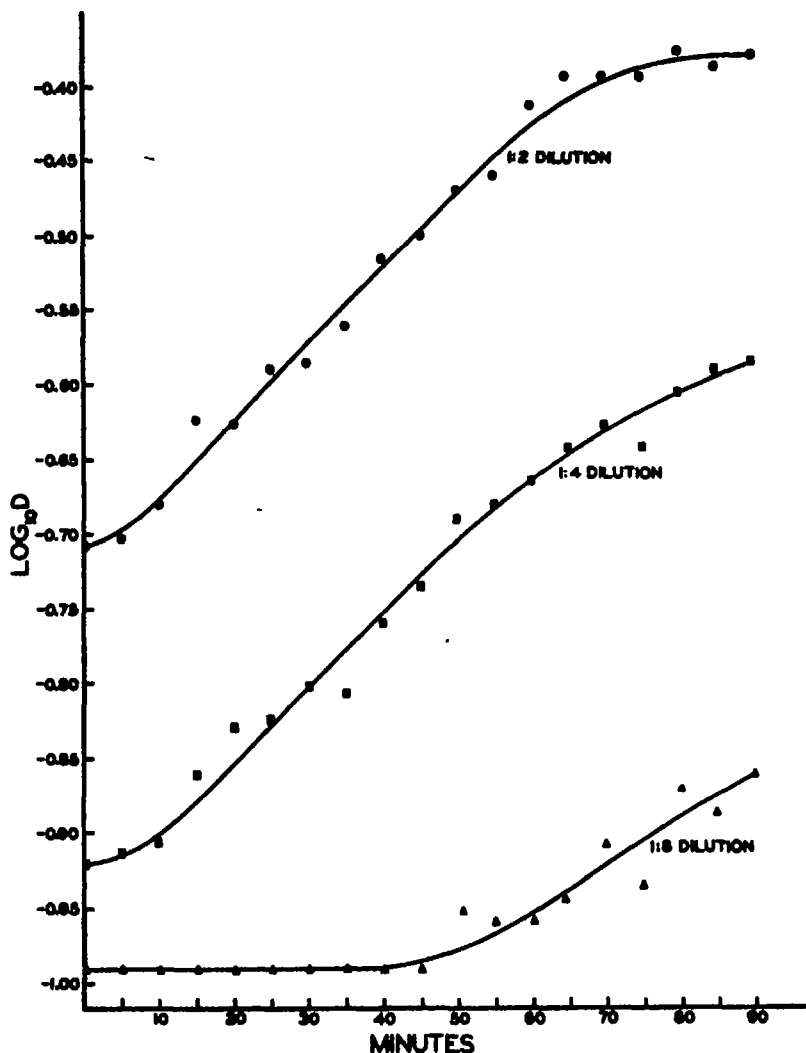
FIGURE 4

Effect of extract dilution on tyrosinase activity of 15,300a extracts.

mean activity index of 15300a is significantly larger than 1 ($t = 4.8$, $df = 4$, $p < 0.01$), while the mean activity of 15300A differs from 1 only with doubtful significance ($t = 2.7$, $df = 4$, $p = 0.05$).   The difference

between the two mating types, as tested by paired comparisons, is highly significant ($t = 8.1, df = 4, p < 0.001$).

While these data do not necessarily prove that monophenol oxidase and polyphenol oxidase are identical, they do demonstrate that if there are two separate, distinct enzymes they are closely associated in 15300a.

Although 15300A does not contain an enzyme capable of oxidizing either tyrosine or dopa, there exists the possibility that this mating type is capable of synthesizing substrate which can be oxidized. In order to investigate this point one portion of 15300a supernate was mixed with an equal volume of buffer solution, while another portion was mixed with an equal volume of 15300A supernate. The mixtures were incubated at 37.5°C. for 2 hours, at the end of which time colorimeter readings were

TABLE 1

Dopa Oxidase Activity in Homogenates of 15300a and A

EXPT. NO.	ACTIVITY INDEX	
	15300A	15300a
1	0.86	1.71
2	0.94	1.80
3	1.00	2.22
4	0.82	1.57
5	0.73	1.31
Mean =	0.87 ± 0.05	1.72 ± 0.15

TABLE 2

Formation of Oxidizable Substrate by the Tyrosinaseless Mating Type (15300A)

EXPT. NO.	INCREASE IN OPTICAL DENSITY	
	CONTROL	15300A + 15300a
1	0.082	0.098
2	0.068	0.084
3	0.094	0.108
4	0.094	0.118
Mean =	0.0845	0.102

made. The results of four such experiments are presented in table 2 and it should be noted that in each experiment the increase in optical density of the mixture of two homogenates was greater than that of the mixture of 15300a homogenate and buffer. The difference between these two, as tested by paired comparisons, is highly significant ($t = 7.95, df = 3, p < 0.01$). The higher optical density resulting from the addition of inactive 15300A supernate to 15300a supernate indicates the presence of suitable substrate in the former. Thus, 15300A synthesizes substrate for which it is incapable of synthesizing an oxidizing enzyme.

These latter data also make it improbable that 15300A supernates fail to exhibit tyrosinase activity because of the presence of an inhibitor rather than the absence of an enzyme, as in the case for the pantothenicless

mutant.[13] The addition of 15300A supernates to 15300a supernates has no apparent inhibitory effect on the latter.

A final observation seems important. Seitz filtration of 15300a supernates results in marked reduction of tyrosinase activity. This reduction of activity can be attributed either to adsorption of the enzyme on the filter or to an association of the enzyme with small particles, not thrown down by the centrifuge but filtered out by the Seitz filter. The latter possibility is particularly attractive, since the association of tyrosinase activity with submicroscopic particles has previously been demonstrated.[13]

Discussion.—The presence of a tyrosinase in 15300a and its absence in 15300A constitutes a difference in protein specificity not previously observed for these strains. The association of this difference with the mating-type locus has not, however, been established. The two mating types of 15300 are not known to differ in any major genetic manner (aside, of course, from mating type), but there are probably many minor genetic differences between them. The association of the enzyme difference with mating type is now undergoing genetic analysis.

It is probable that this difference is a genetical one. The genetic control of melanin production in mammals is well understood.[14] In these cases the changes in pigmentation have been demonstrated to be the result of genetically controlled changes in dopa oxidase activity.[10, 15] A similar situation has been demonstrated in the ascomycete *Glomerella cingulata*, where the tyrosinase concerned seems closely similar to the one demonstrated in the present work.[16] In all of these cases many genes have been demonstrated to influence the enzyme, and it seems that the primary effects of some of these are not directly upon the tyrosinase itself.

In the present instance a situation of a different sort obtains. The mating-type difference is itself a specificity difference of a sort, since the organs of both sexes are present in both mating types. In many respects the mating-type reaction seems similar to the phenomenon of pollen incompatibility, which has in turn been noted to be related to immunological reactions in animals.[1, 17] In the strain we have used, the two mating types also differ in enzyme specificity and antigenic specificity. While the association of these latter differences with the mating-type locus has not yet been investigated, we seem to have a case well suited for a critical test of the hypothesis of the unitary nature of primary gene action.

Summary.—1. The two mating types of Strain 15300 (albino-2), *N. crassa*, differ in ability to produce melanin.

2. This difference has been demonstrated to be due to the presence in 15300a of a tyrosinase exhibiting both mono- and polyphenol oxidase activity and its absence in 15300A.

3. Both mating types possess suitable substrate for the activity of this enzyme.

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THE NATURE OF LINKAGE VARIATION WITH AGE IN INVERSION HETEROZYGOTES OF DROSOPHILA MELANOGASTER

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Two kinds of variation in linkage values in Drosophila experiments are well known but poorly understood. One is the change in crossover values with increasing age of the female as shown by Bridges[1]; the other is excessive variation from female to female as pointed out by Gowen.[2] Studies reported in the present paper on an age effect in a special situation may contribute to the interpretation of both problems. They also serve as controls for the irradiation paper which follows.[3]

The chief result of aging is a rapid decrease in recombination values during the first six days of egg-laying, particularly at or near the spindle attachment of the chromosome. After that period smaller changes consist, with variations, of a slight rise and second fall. In recognition of this Bridges[4] has defined as a condition for chromosome mapping the use of

data from young females. From such data inferences are made as to the amount of actual crossing over which has occurred between genes which are assumed to be heterozygous at the time when the germ cells enter meiosis. That this procedure may overestimate the amount of crossing over has been elaborated by the senior author both by reasoning back from observable clustering of the data[3] and by deducing certain consequences of crossing over premeiotically, in gonial cells.[4] The present experiment shows an age effect and clustering attributable to oögonial crossing over in the same body of data, where meiotic crossovers have been greatly reduced or eliminated by inversions.

The age effect in this stock was encountered by accident. In the course of stockmaking by the senior author it was noticed that the infrequent crossovers from al^2 Cy lt^3 L^4 sp^2/S Pfd females appeared in early cultures but not in later transfers. Of 14 crossovers in the Cy-L interval, 12 were found in the first two cultures and none at all in the last two of a series of five transfers. It was not clear whether this was an accidental result until a larger experiment had been carried out by the junior author and a genuine age effect found.

The experiment was planned with several improvements on the original mass matings. By testcrossing to light males use was made of the lt locus, which differentiated the crossovers into two regional kinds. The map order of the loci, the number designations of the crossover regions and the extent of the inversions may be diagrammed as follows:

$$
\begin{array}{llll}
S & & & Pfd \\
\hline
-Cy----- & lt3 & -----L^4--- \\
1 & 2 & 3 & 4
\end{array}
$$
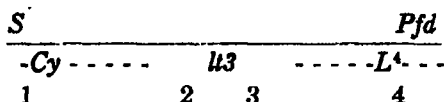
The spindle attachment lies to the right of lt. In the experiment, females were mated individually to these light males, so that the contribution of each family to the totals would be known. Furthermore, the data for each family came separately from five consecutive coded cultures which were not decoded and summed as to families until the experiment was over.

Results and Discussion.—This experiment confirmed the age effect which had been encountered in mass mating. Hence data from both tests have been combined in table 1. In the first two cultures crossovers comprised about 1% of the offspring, in the third they were 0.25% and in the last two transfers practically zero. Almost all of the crossovers came from eggs laid during the first five days of adult life. A chi-square test showed that this was a highly significant departure from the mean of all 11 days. We were therefore dealing with an age effect exhibited by these crossovers.

The data were next examined family by family to see whether the crossovers had appeared at random or in clusters. Random distribution would be in accord with the usual assumption that crossing-over occurs at meiosis,

meaning that no more than one crossover chromatid from each tetrad is recoverable by breeding. Clustering would indicate that there was some gonial influence[7] either (a) completed crossing-over in a gonial cell, or (b) weakening of a certain place in a chromosome followed later by crossingover in identical regions in numerous related primary oöcytes. Table 2 shows agglutination of the data, as mathematicians call it, after adding up the cases of crossovers in the two regions of the eighteen families. Although a majority of the 36 cases had no crossovers, paradoxically threefourths of the crossovers appeared in groups of two or more, as if from the same event of exchange. Furthermore the cases of solitary crossovers, 8, seem too low for a Poisson distribution. Some probabilities will be presented in later paragraphs.

TABLE 1

SUMMARY OF SINGLE CROSSOVERS RECOVERED FROM *Star Pufdi/Curly light Lobe*[4] INVERSION HETEROZYGOTES IN BOTH EXPERIMENTS ACCORDING TO AGE OF FEMALES IN DAYS

Cultures	1	2	3	4	5
Age of female	0–3	3–5	5–7	7–9	9–11
Crossovers	17	20	4	1	1
Total offspring	1747	2062	1677	1752	1886

TABLE 2

AGGLUTINATION IN THE FINDING OF SINGLE CROSSOVER FLIES, FOR EITHER REGION, AMONG 18 FAMILIES OF *S Pfd/Cy lt L*[4] INVERSION HETEROZYGOTES

CROSSOVERS/FAMILY	CASES	TOTAL CROSSOVERS
0	19	0
1	8	8
2	7	14
3	1	3
4	1	4
Totals	36	29

NOTE: Inclusion of seven multiple crossovers would increase the numbers of pairs and especially of triplets.

More detailed familial data appear in table 3. The smallest family, No. 1, contained 4 crossovers in region 2, and these were not all from the same culture, so that they tend to confirm each other. The next smallest family had 3 crossovers in region 3, all found in the same culture. In seven other cases 2 crossovers of a kind were found, and more often than not these were in separate cultures rather than together. Many families, representative in size, failed to have any crossovers in one or both regions. Because of this clustering the crossover values showed great variability among the different families, from about 5 per cent to zero.

One may compute the probability that these are merely chance deviations

from randomness.   In region 2 the over-all frequency of crossovers is approximately 0.002, (15/7534 from table 3).   In ten families having some 4700 offspring no such crossovers were found, and the probability of this as a random result is 0.998^{4700}, or 0.00008.   On the same hypothesis, the probability of drawing a sample of 108 including as many as 4 similar crossovers (Family 1) is 0.0008.   Then the joint probability of occurrence of these two aspects of the experiment is 6.4×10^{-8}.   Inclusion of the fact that pairs of crossovers were found more often than singles would make it even more improbable that the crossovers in region 2 were independently determined. ·

TABLE 3

SUMMARY OF TESTCROSS DATA FROM 84 FERTILE, CODED CULTURES AS REASSEMBLED INTO THE ORIGINAL 18 FAMILIES AFTER CLASSIFICATION.   FAMILIES LISTED IN ORDER OF INCREASING SIZE

FAMILY NO.	TOTAL PROGENY	SINGLE CROSSOVERS REGION 2	REGION 3	MULTIPLE CROSSOVERS
1	108	4*	1	
2	135	1	3	
3	268	1	0	
4	387	0	1	
5	408	1	2*	
6	417	2	0	
7–11	5 × 439.4	0	0	
12	445	2*	2	
13	481	0	0	S Cy;* lt, wild.
14	490	2*	1	
15	520	0	2	Cy L
16	533	2*	1	
17	533	0	1	S lt Pfd
18	612	0	0	S lt Pfd L;* L,
Totals	7534	15	14	7

* Denotes crossovers obtained from two different coded cultures.

Similar calculations for region 3 show that events there are very far from random.   The probability that families containing a total of 3975 offspring would have no crossovers, if the latter were randomly distributed at a frequency of 0.183%, is 0.99817^{3975}, which is less than 0.0006.   Turning to the other end of the distribution we find that the probability of getting as many as 3 crossovers in a family of only 135 offspring is 0.0157 on the same assumption.   The joint probability for the two extremes is more remote, and the further joint probability for region 2 crossovers and region 3 crossovers is 6×10^{-11}.

Up to this point the negative aspects of data of table 3 have been discussed.   The kind of non-randomness pointed out may be explained by a

limited amount of gonial crossing over plus gonial multiplication. While this is perhaps the better explanation, there is another interpretation which could reserve crossing over until meiosis. Thus a tendency to produce crossovers near the spindle attachment regardless of region may be possessed by some heterozygotes more than by others. In table 3 one may see that pairs and larger clusters in one region are almost always accompanied by at least one crossover in the adjacent region. This is true in 6 out of 8 families; only females No. 6 and No. 15 had 2 crossovers in one region without any single exchanges in the other. Yet a double crossover, a Curly Lobe fly, appeared in family No. 15 in the same culture with 2 Star Lobe single crossovers. This makes 7 cases of crossing over adjacent to the regions represented by the 8 clusters.

Further evidence for this alternative may be obtained from the multiple crossovers as a whole, which have therefore been included in table 3, even though they are not fully understood. The most plausible multiple crossovers are the Curly Lobe fly just mentioned and the Star light Pufdi of family No. 17. These could be region 2–3 double crossovers, and each was formed in a female which also had one or two single crossovers in region 3. The expectation for these double crossovers in an entire experiment of this size is less than one fly even if figured on the basis of the initial high point of recombination values, 1%, and with no interference. The other multiple crossovers are far less likely than the preceding. The finding of a wild and a light fly, the latter verified by breeding, in the same culture of family No. 13 might be explained as contamination; or these might be triple crossovers, or even quadruples, in some way related to a Star Curly quadruple crossover independently classified in a previous culture. In the subsequent experiment in which similar heterozygotes were irradiated (Hinton and Whittinghill[3]), two more light phenotypes were found in different cultures, so it remains a possibility that this lt fly was a 1-2-4 triple crossover. It is indeed strange that there were no single crossover offspring in this family which had two different triples and a quadruple crossover. In one other family, 18, single crossovers were lacking. The only crossovers in this the largest family of all, were a $S\ lt\ Pfd\ L^4$ 2–3–4 triple and a verified L^4 1–3 double. Neither kind was detected in the next experiment. However the sporadic nature of the multiple crossovers may be viewed as an extension of the non-randomness already demonstrated among the single crossover types of regions 2 and 3. No single crossovers were recovered from regions 1 or 4 due to the inversions there.

Summary.—There is a decrease in the frequency of crossover offspring from $S\ Pfd/Cy\ lt\ L^4$ females as they age. Crossover values drop practically to zero in seven days, in agreement with the previously known decrease from normal females. Variability in crossover values from family to family was greater than one would expect by chance if each crossover

had been formed separately and independently of every other crossover, the usual assumption about meiosis.[8] Two better explanations have been considered. An entire cluster of crossovers may represent only one actual crossing over in an oögonial cell which was the common ancestor of several eggs. Such an hypothesis is preferred, but an alternate explanation may fit the data of this one experiment without recourse to gonial crossing over. The clustering may begin with a gonial conditioning, such as a permanent weakening of a chromosome, which may influence the location of several later meiotic crossings over. Because identical crossovers and adjacent crossovers tended to be found in the same families in this favorable stock of flies, either of the new hypotheses is appropriate for the present data. Great variability of spontaneous crossover values near spindle attachments would seem to be inescapable in experiments based on the early broods prescribed by Bridges. Hence the collection of data for maps might well be done from a later egg-laying period and, particularly, from large numbers of females to get a measure of the variability.

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THE DISTRIBUTION OF X-RAY INDUCED CROSSOVERS FROM CURLY INVERSION HETEROZYGOTES OF DROSOPHILA MELANOGASTER FEMALES

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Introduction.—That crossing over may occur in oögonial cells was suggested by the results of experiments by Whittinghill[1] in which crossing over was induced by x-rays in the X-chromosomes of Drosophila females homozygous for the *c3G* asynaptic factor. It had previously been demonstrated that induced crossovers recovered from Drosophila males were of spermatogonial origin.[2, 3] Cooper's observations[4] of chiasmata in gonial cells of both sexes of Drosophila may provide cytological foundation for the hypothesis of gonial origin of some crossovers, particularly those which are x-ray induced. Somatic crossing over, the basis for the occurrence of twin spots in the hypodermis of Drosophila males and females,[5] closely parallels gonial crossing over in the formation of daughter cells which have become homozygous distally. The possible consequences of oögonial crossing over upon linkage data, as recently discussed by Whittinghill,[6] illustrate the need for further investigation of this phenomenon. This paper reports the results of x-ray induced crossing over in Curly inversion heterozygotes of *Drosophila melanogaster* females, and these results are interpreted in relation to normal, or random, meiotic events as opposed to oögonial events of recombination.

Experimental Methods.—Stocks and experimental procedures, with the exception of the treatment employed in this work, were identical with those described in the companion paper[7] in these PROCEEDINGS. Twenty-eight adult virgin females between six and twelve hours old were placed in a 0.7-ml. gelatin capsule and exposed to a total x-ray dose of 2250 r, which was received at the rate of 350 r per minute from a distance of 20 cm. through a 4-mm. aluminum filter from a tube operating at 140 kv. These females were allowed to feed for one day before being testcrossed separately to 4 or 5 *lt/lt* males in consecutive half-pint bottles for life. Transfers, without etherization, were made every other day for the first seven cultures and at longer intervals for the final three cultures. As in the former experiment, all cultures were coded so that classifications were performed without bias. Uncertain phenotypes were determined by testcrosses to light homozygotes. Although the spontaneous recombination data of the preceding report have been employed to some extent as controls, accurate comparison of the frequency of crossing over is not possible because of the absence of randomly determined recombination offspring.

The authors have concentrated their study on the actual distribution of induced recombinants in an attempt to detect the place and manner of their origin.

Results.—Although fertility of the irradiated adults was decidedly lower than that of controls, recombination values were greatly increased throughout the egg-laying period (table 1). This persistence of the increase in recombination for as long as 31 days after treatment may have been the result of early as well as late oögonial cells having been affected. Any influence of age on spontaneous recombination is obscured by the much larger effect of the x-rays. This effect included a real peak in recombination between 4 and 8 days after treatment, as shown by the standard errors for cultures 3 and 4 in table 1.

TABLE 1

COMPARATIVE EFFECTS OF AGE (C) AND X-RAYS (X) ON RECOMBINATION VALUES IN
CONSECUTIVE TESTCROSS CULTURES OF S Pfd/Cy lt L⁴ FEMALES

CULTURE NUMBER	FERTILE FEMALES (C)	FERTILE FEMALES (X)	TOTAL PROGENY (C)	TOTAL PROGENY (X)	PER CENT RECOMBINATION (C)	PER CENT RECOMBINATION (X)
1	18	20	1334	60	0.82 ± 0.24	5 00 ± 2 81
2	18	21	1681	532	0.83 ± 0.22	3.95 ± 0.27
3	18	16	1414	250	0.14 ± 0.10	10.80 ± 1.96
4	18	14	1511	549	0.07	7.47 ± 1.12
5	18	13	1594	379	0.06	5.01 ± 1.12
6		13		631		3.65 ± 0.75
7		12		467		1.93 ± 0 64
8		9		390		2.82 ± 0.84
9		6		427		4.22 ± 0.97
10		1		34		11.76 ± 5.52
Totals			7534	3719	0.38 ± 0.07	4.73 ± 0.35

Three results which are unexpected in random meiotic processes are revealed when the data are recombined into familial groups (table 2). The first notable feature of the table consists of large deviations from the mean total recombination value of 4.73 ± 0.35 per cent. These values range from zero to above 12 per cent. Secondly, the usual assumption of linearly random exchanges is not substantiated due to the wide variations in proportions of region 2 to region 3 crossovers as exhibited chiefly in the families of Females 5, 11 and 13, as compared with 16. Thirdly, pronounced inequalities in complementary crossover classes within a region may be observed in the crossover columns of Females 5, 11, 16 and 23. All of these three forms of variation from female to female have been shown to be expected consequences of oögonial crossing over[1, 6] and may be interdependent to some extent. The most diagnostic of these consequences, and the one which lends itself most readily to analysis, is the extent of imbalance between complementary crossover classes.

TABLE 2

FAMILIAL DISTRIBUTION OF TESTCROSS OFFSPRING FROM IRRADIATED ADULT
$S Pfd/Cy \ lt \ L^4$ FEMALES

FEMALE NUMBER	NON-CROSSOVERS		SINGLE CROSSOVERS				TOTAL PROGENY	PER CENT RECOMBINATION
	$S Pfd$	$Cy \ lt \ L^4$	REGION 2		REGION 3			
1	96	96	3	0	2	2	201	3.48 ± 1.29
2	128	146	5	2	5	2	288	4.86 ± 1.27
4	86	95	2	3	3	0	189	4.23 ± 1.46
5	274	261	13	7	0	0	556°	3.96° ± 0.83
8	120	135	1	1	1	0	258	1.16 ± 0.67
11	103	85	8	2	0	1	199	5.53 ± 1.62
12	33	25	3	3	1	1	66	12.12 ± 4.03
13	135	136	9	10	4	2	296	8.45 ± 1.62
15	7	8	0	0	1	0	16	6.25 ± 6.06
16	225	233	1	1	12	5	477	3.98 ± 0.90
18	238	230	5	7	6	5	491	4.68 ± 0.95
19	62	59	4	2	2	0	129	6.20 ± 2.12
20	36	49	0	1	1	0	87	2.30 ± 1.67
22	21	33	3	1	1	3	62	12.90 ± 4.26
23	58	62	0	2	5	0	127	5.51 ± 2.02
26	85	91	5	2	2	1	186	5.38 ± 1.65
12 others	39	52	0	0	0	0	91	0
Totals	1746	1798	62	44	46	22	3719	4.73 ± 0.35

° Includes 1 $S \ lt \ Pfd$ double crossover.

TABLE 3

CHI SQUARE ANALYSIS OF THE DISTRIBUTION OF COMPLEMENTARY CROSSOVERS WITHIN
FAMILIES OF IRRADIATED FEMALES

FEMALE NUMBER	REGION 2			REGION 3		
	$S \ lt \ L^4$: $Cy \ Pfd$	p	$-\text{Log}_e$	$S \ L^4$: $Cy \ lt \ Pfd$	p	$-\text{Log}_e$
1	3:0	0.125	2.080	2:2	0.500	0.693
2	5:2	0.227	1.483	5:2	0.227	1.483
4	2:3	0.500	0.693	3:0	0.125	2.080
5	13:7	0.132	2.025	(0:0)	...	....
8	1:1	0.500	0.693	(1:0)	...	....
11	8:2	0.055	2.904	(0:1)	...	....
12	3:3	0.500	0.693	1:1	0.500	0.693
13	9:10	0.500	0.693	4:2	0.344	1.067
16	1:1	0.500	0.693	12:5	0.072	2.632
18	5:7	0.387	0.950	6:5	0.500	0.693
19	4:2	0.344	1.067	2:0	0.250	1.386
22	3:1	0.313	1.161	1:3	0.313	1.161
23	0:2	0.250	1.386	5:0	0.031	3.478
26	5:2	0.227	1.483	2:1	0.500	0.693
Total $-\text{Log}_e$			18.004			16.059
Chi-square	(28 d. f.)		36.008	(22 d. f.)		32.118
Probability			0.154			0.079

Joint Probability = 0.0121

In order to answer the question whether the observed deviations from equality are greater than those allowed by chance, the following analysis was performed. Probabilities of obtaining the observed distribution of complementary crossover classes in each family or of any distribution deviating more in the same direction were computed (table 3). The $-\log_e$, equal to $chi^2/2$ associated with two degrees of freedom, is additive in character,[8] and this allows an over-all probability to be obtained for either region for all families. Since the two regions concerned are on opposite sides of the centromere, one may assume that crossings over occurring simultaneously in these regions are independent of each other. Distributions of 1 : 0 or 0 : 1 have been omitted from calculations, for although they add two degrees of freedom to the total, they can contribute no useful information here.

While there are no individual probabilities in the above table which are significant alone, there appears to be a definite excess of distributions of low likelihood as shown by the probabilities of 0.154 and 0.079 for regions 2 and 3, respectively, all families combined. Furthermore, the joint probability for both regions is 0.0121, so that a similar over-all distribution may be expected to occur by chance alone only one time in one hundred like experiments.

Viability differences in crossover chromosomes may sometimes account for numerical differences in complementary crossover classes throughout an experiment; however, this possibility may be reasonably eliminated here. Six additional testcrosses of males whose genotypes corresponded to a combination of complementary crossover chromosomes from region 2 exchanges yielded 706 *S lt L*[4] and 792 *Cy Pfd* flies. This inequality is in the opposite direction from that in the crossover progeny from testcrossed females which gave a 62:44 majority to the *S lt L*[4] class. As there is no reason to suspect that the relative viabilities of the two chromosomes may be reversed depending on whether they originate from sperms or eggs, some cause other than viability differences must be responsible for the observed inequalities in complementary crossover offspring from irradiated females. Similar tests yielding 579 *SL*[4] : 530 *Cy lt Pfd* indicate that larval viability differences played little or no part in the 46:22 result from region 3.

Previous studies of induced crossing over in Drosophila have revealed a tendency of some recombinants to appear simultaneously among the testcross progeny, and several instances of this irregularity occurred in this experiment. A cluster of 6 region 2 recombinants distributed equally was recovered from the third culture of Female 13 among a small brood of 19 flies which included only one crossover from the third region. By comparison, the mean brood size per fertile culture was found to be 33.50 of which 1.58 flies would be expected to be recombinants, assuming a ran-

dom distribution. The fourth culture of Female 4 contained only 3 recombinants, identical region 2 triplets, among a total brood of 42. At this same age, Females 11 and 18 each produced identical quadruplets from region 2, and each set was accompanied by twins of the complementary class; in addition, the latter female had twins from region 3. While these examples are from the two cultures which exhibited the maximum recombination values, clustering was not limited to this period. It is very unusual that although Female 5 produced 7 region 2 recombinants out of 182 offspring in Culture 9 (20–26 days), region 3 recombinants were not represented; the Culture 10 (27–31 days) brood of 34 from this same female included 4 crossovers, all from region 2. Other clusters of lesser magnitude might be cited, and it is possible that some clusters were missed due to splitting them when transferring the cluster-producing female into a new culture.

Two light homozygotes, classified with the broods of Females 18 and 23, have not been included in the data because of the possibility of their being contaminants. Yet, they are not too improbable representatives of quadruple crossing over when compared with similar multiple crossovers which appeared in controls. Furthermore, in order to recover any recombinants from a region included in an inversion, at least double crossing over must occur since single exchanges alone lead to the production of lethal dicentrics and acentric chromosome fragments.[9] One is also reminded that although the Curly inversions do severely limit the frequency of single exchanges in adjacent non-inverted regions, the non-random occurrence of recombinants resulting from such exchanges is not explained by the presence of these inversions.

Discussion.—If an exchange of homologous segments of paired bipartite prophase chromosomes should occur in only a relatively few oögonial cells, and the products of such an exchange be erratically reproduced before the initiation of meiosis, one may well expect the resulting recombination offspring to be distributed non-randomly as recorded in this experiment. Or, as suggested by the distribution of spontaneous recombinants from Curly inversion heterozygotes, oögonial *exchange* of homologous segments may not be necessary for agreement with the results if only a localized weakening of one chromatid at this time may predispose meiotic crossing over at this point after several mitotic divisions have intervened. It seems plausible that these alternatives could be tested by a cytological examination of the first meiotic division, for if exchange occurred prior to this division, the regions distal to the break and including the inversion would frequently become homozygous, allowing normal synapsis in this arm and free chiasma formation.

On either hypothesis, it is necessary to assume unequal multiplication of the affected chromosomes, and the agencies responsible for this may be

several. For example, different rates of division might be conditioned by the exchange products themselves, or could be imposed by chance according to the relative positions the daughter oögonia occupy in the ovary; some of them may be crowded aside from the main line of division either to be lost entirely or relegated to later appearance. However, some causal factor other than larval viability differences must operate preferentially in one direction in order to explain the total inequalities in complementary crossover adults. Cell lethals could presumably explain this if some oögonia become homozygous due to prior exchange.

Applying this idea to the present experiment, we may assume that Star and Curly may become homozygous in adjacent cells after region 2 exchanges, and that Pufdi and Lobe can become homozygous after region 3 exchanges. If any of these mutants are homozygous cell lethals (all are homozygous lethal in larvae), they could not cause more than a 50 per cent reduction of any crossover chromosome, since such chromosomes may segregate into heterozygous situations as frequently as into the homozygous condition. We would then infer from the 46 $S\ L^4$:22 $Cy\ lt^3\ Pfd$ ratio of recombinants that Pufdi is completely lethal to cells homozygous for this factor; i.e., in $S\ Pfd/Cy\ lt\ Pfd$ gonia. In the case of region 2 exchanges, Curly would appear to be detrimental, although not completely lethal, to oögonial cells homozygous for it as judged from the ratio of 62 $S\ lt^3\ L^4$ to 44 $Cy\ Pfd$ recombinants from this region. While this hypothesis is dependent on oögonial exchange, other possibilities may be found which agree equally well with oögonial weakening and subsequent meiotic crossing over.

Two hypotheses of the mode of action of x-rays in inducing crossing over have appeared in the literature, and these may be briefly considered. In attempting to demonstrate a single mechanism of crossing over for both male and female Drosophila, Friesen[10] found that the Curly inversions did not limit x-ray induced crossing over between black and cinnabar in males as much as in females. On the basis of this evidence, the lack of spontaneous male crossing over was ascribed to an incomplete conjugation of homologous chromosomes (the greater the normal degree of synapsis, the greater the depressing influence of the inversions). Friesen further explained the action of x-rays while increasing recombination to result ih "a closer intercourse of the long autosomes (first of all in the central regions)" for both sexes. This hypothesis obviously fails to account for the nonrandom occurrence of recombinants in our experiments, unless it is applied to the chromosomes of gonial cells. Friesen's omission of this application apparently indicates abandonment of his own earlier proposal that crossovers induced in males were of spermatogonial origin.

Another possible mode of action of x-rays in inducing crossing over which more nearly conforms to our experimental results has recently been ad-

vanced by Cooper[4] as a result of observations of chiasma-like configurations in Drosophila gonial cells of both sexes. While it is clear that such chiasmata do not regularly lead to spontaneous recombination, at least in the male, Cooper supposes that these configurations may be competent targets for ionizing particles, and he concludes that x-ray induced crossovers are probably of gonial origin. This would be the place of origin of the crossovers found in this experiment in view of the tendency for complementary crossovers to vary in either direction from the expected.

Summary.—The induced crossover offspring from testcrossed *S Pfd/Cy ll³ L⁴* females x-rayed with 2250 r as adults were so distributed that their meiotic origin is unlikely. The effects of the x-rays were evident for as long as 31 days, with a peak in recombination between 4 and 8 days after treatment. Wide variations from female to female were observed in total per cent recombination, in the relative numbers of recombinants from adjacent regions, and in the distribution of complementary crossover classes within each region. Some tendency toward clustering of the recombinants among the testcross offspring was revealed. These results are indicative of some oögonial influence upon recombinants. This influence may be merely a weakening of oögonial chromosomes followed much later by meiotic crossing over at the weak point, or it may be completion of crossing over in the oögonia. The data on the variable balance of complementary classes favors the latter hypothesis.

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VARIOUS KERNELS IN THE THEORY OF PARTIAL DIFFERENTIAL EQUATIONS*

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1. We deal with the partial differential equation

$$\Delta u = q(P)u, \qquad \Delta = \frac{\partial^2}{\partial x^2} + \frac{\partial^2}{\partial y^2} \qquad (1)$$

where $q(P)$ is a positive continuously differentiable function of the point $P = (x, y)$ in the closure of a finite domain D of the x, y-plane. We suppose that D is bounded by a set C of analytic curves. We define the bilinear integral

$$E\{u, v\} = \int\!\!\int_D [\text{grad } u \cdot \text{grad } v + q\, uv] d\tau, \quad d\tau = \text{area element in } D \quad (2)$$

and observe that $E(u) = E\{u, u\}$ is the energy integral connected with (1).

Let Σ be the linear space of all solutions of (1) with finite energy integral; we may introduce into Σ the metric based on the scalar product (2) and consider Σ as a Hilbert space. Let $N(P, Q)$ and $G(P, Q)$ denote Neumann's and Green's functions of the differential equation (1) with respect to D. One shows easily that for each function $u \in \Sigma$ there holds:

$$E\{N(P, Q), u(P)\} = u(Q), \qquad E\{G(P, Q), u(P)\} = 0. \qquad (3)$$

Here and in the following operations, the E-multiplication is always to be understood with respect to the letter common to both factors.

We define the two kernels

$$K(P, Q) = N(P, Q) - H(P, Q),$$
$$L(P, Q) = N(P, Q) + G(P, Q). \qquad (4)$$

$K(P, Q)$ is as function of each variable P an element of Σ; in view of (3) it satisfies the identity

$$E\{K(P, Q), u(P)\} = u(Q), \qquad u \in \Sigma, \qquad (3')$$

i.e., it is the reproducing kernel of the class Σ. By Schwarz' inequality

$$u(Q)^2 \leq K(Q, Q) \cdot E(u), \qquad (5)$$

i.e., the kernel K provides at each point $Q \in D$ an estimate for the value of u in terms of its norm in Hilbert space. The inequality is the best possible, equality holds for $u(P) = K(P, Q)$. If a set of functions $\{u_r(P)\}$ forms a

complete orthonormal set in Σ, we can develop $K(P, Q)$ into the Fourier series

$$K(P, Q) = \sum_{\nu=1}^{\infty} u_\nu(P)u_\nu(Q). \tag{6}$$

This property provides a useful numerical approach to the theory of Green's and Neumann's functions [1, $a - d$].

The kernel $L(P, Q)$ has also the reproducing property under scalar multiplication. It does, however, not belong to Σ because of its singularity for $P = Q$ and has, therefore, not the same importance in the theory of orthogonal solutions of (1). It was originally introduced for reasons of formal symmetry. See reference 3. Its real significance will be shown in the sequel.

2. Let $D_1 \supset D$ and suppose that $q(P)$ is positive and continuously differentiable in D_1. Denote by N_1, G_1, K_1, L_1 the corresponding Neumann's and Green's functions and their combinations with respect to the domain D_1. Let for $P, Q \in D$

$$k(P, Q) = K(P, Q) - K_1(P, Q),$$
$$l(P, Q) = L(P, Q) - L_1(P, Q); \tag{7}$$

denote finally by D_0 the difference region between D_1 and D and let $E_0\{u, v\}$ be the integration defined in (2) but extended over D_0. With these notations, we state the following result which is an easy consequence of the boundary behavior of Green's and Neumann's functions:

$$E\{l(P, Q), l(P, R)\} = k(Q, R) - E_0\{L_1(P, Q), L_1(P, R)\}. \tag{8}$$

Let P_i be an arbitrary set of N points in D and x_i be N arbitrary real numbers. We derive from (8) the identity

$$\sum_{i,k=1}^{N} K(P_i, P_k)x_ix_k - \sum_{i,k=1}^{N} K_1(P_i, P_k)x_ix_k =$$
$$E_0\{ \sum_{i=1}^{N} L_1(P, P_i)x_i\} + E\{ \sum_{i=1}^{N} l(P, P_i)x_i\}. \tag{8'}$$

Since the right-hand side of (8') is non-negative we conclude that the quadratic forms based on the K-kernels decrease monotonically with increasing domain.

Suppose that for the domain D_1 the fundamental functions N_1 and G_1 are known. In this case, (8') leads to an estimate for the unknown kernels of all subdomains $D \subset D_1$:

$$\sum_{i,k=1}^{N} K(P_i, P_k)x_ix_k \geqq \sum_{i,k=1}^{N} K_1(P_i, P_k)x_ix_k + E_0\{ \sum_{i=1}^{N} L_1(P, P_i)x_i\} \tag{8''}$$

where all right-hand terms are known.

3. The kernel $K(P, Q)$ becomes logarithmically infinite if both argument points tend to the same point P_0 on the boundary C of D. The same holds then for the kernel $k(P, Q)$ since $K_1(P, Q)$ is continuous in the interior of D_1. It can be shown that $l(P, Q)$ is continuously differentiable in the closed region $D + C$. It thus appears that among the difference kernels k and l, the l-kernel is the more regular while among the K and L-kernels the K-kernel has the better regularity character. By means of identity (8), we are now able to investigate easily the singularity of $k(P, Q)$ on the boundary C of D. In fact, $k(Q, R)$ and $E_0\{L_1(P, Q), L_1(P, R)\}$ have there the same asymptotic behavior, since their difference is regular in the closed region.

The linear transformation of the Hilbert space Σ into itself

$$T_u(P) = E\{l(P, Q), u(Q)\} \qquad (9)$$

can be shown to be completely continuous. Thus, the eigenvalues of the integro-differential equation

$$u'_\rho(P) = \lambda_\rho E\{l(P, Q), u_\rho(Q)\} \qquad (10)$$

form a discrete spectrum. If we also permit the eigenvalue $\lambda = \infty$, we can select a system of eigenfunctions $\{u_\rho(P)\}$ of (10) which form a complete orthonormal set in Σ. In terms of this set, we have the Fourier developments

$$K(P, Q) = \sum_{\rho=1}^{\infty} u_\rho(P)u_\rho(Q), \qquad l(P, Q) = \sum_{\rho=1}^{\infty} \frac{1}{\lambda_\rho} u_\rho(P)u_\rho(Q), \qquad (11)$$

converging uniformly in each closed subdomain of D. It can be shown that all eigenvalues λ_ρ satisfy the inequality

$$|\lambda_\rho| > 1. \qquad (10')$$

4. We assume again that the fundamental functions of D_1 are known. Then the expression

$$K_1(Q, R) + E_0\{L_1(P, Q), L_1(P, R)\} = \Gamma(Q, R) \qquad (12)$$

is to be considered as an elementary quantity, obtainable from N_1 and G_1 by the processes of algebra, differentiation and integration. We can reduce the solution of the boundary value problems with respect to (1) and the domain $D \subset D_1$ to sequences of such elementary operations.

We remark from (8) and (11) that

$$M(Q, R) = K(Q, R) - \Gamma(Q, R) = \sum_{\rho=1}^{\infty} \frac{1}{\lambda_\rho^2} u_\rho(Q)u_\rho(R). \qquad (13)$$

This shows that the positive-definite kernel $M(Q, R)$ has its lowest eigenvalue larger than 1. Let us consider the integral equation for $K(Q, R)$ with kernel $M(P, R)$.

$$K(Q, R) - E\{M(P, R), K(P, Q)\} = \Gamma(Q, R) \qquad (14)$$

which is an immediate consequence of the reproducing property of the K-kernel. Since the lowest eigenvalue of M is larger than 1 we can solve (14) by means of the Neumann series development:

$$K(Q, R) = \sum_{\nu=0}^{\infty} E\{M^{(\nu)}(P, Q), \Gamma(P, R)\} \qquad (15)$$

where the iterated kernels $M^{(\nu)}(P, Q)$ are defined by

$$M^{(\nu)}(P, Q) = E\{M^{(\nu-1)}(T, P), M(T, Q)\},$$
$$M^{(0)}(P, Q) = K(P, Q). \quad (15')$$

Introducing similarly the νth iterated kernel $\Gamma^{(\nu)}(Q, R)$ based on $\Gamma^{(1)} = \Gamma$ as defined by (12), we may transform (15') by means of (13) into

$$K(Q, R) = \sum_{\nu=0}^{\infty} \left[\sum_{\rho=0}^{\nu} \binom{\nu}{\rho} (-1)^\rho \Gamma^{(\rho+1)}(Q, R) \right]. \qquad (16)$$

Thus, $K(Q, R)$ is developed into an infinite series of terms each of which can be obtained by elementary processes. Let $K_n(Q, R)$ be the kernel obtained by summing in (16) only for $0 \leq \nu \leq n$; the difference kernel $K - K_n$ can be shown to be positive-definite so that each approximate calculation of K leads to an inequality for the K-kernel. (8″) is the inequality obtained by taking $n = 0$.

5. Let us suppose that the curve system C is obtained from the analytic boundary curves C_1 of D_1 as follows: If s is the length parameter of C_1, we define an analytic non-negative function $\varphi(s)$ on C_1; we erect at each point $P_1 \epsilon C_1$ the interior normal and proceed on it by an amount $\epsilon\varphi(s) = \delta\nu$; where $\epsilon \leq 0$ is a smallness parameter. The end-points $P(s)$ thus obtained form, for ϵ small enough, a permissible C system. We can show that for all eigenvalues of (10)

$$\left| \frac{1}{\lambda_\nu} \right| = O(\epsilon) \qquad (17)$$

holds. This implies by (13)

$$M(Q, R) = O(\epsilon^2), \qquad M^{(\nu)}(Q, R) = O(\epsilon^{2\nu}), \qquad (18)$$

uniformly in each closed subdomain of D.

Using the estimate (18) in (16), we find after elementary transformations

$$K(Q, R) - K_1(Q, R) = \int_{C_1}[\text{grad } K_1(P, Q)\cdot \text{grad } K_1(P, R) +$$
$$q(P)K_1(P, Q)K_1(P, Q)]\delta\nu_P ds_P + O(\epsilon^2) \quad (19)$$

where the estimate $O(\epsilon^2)$ holds uniformly in each closed subdomain of D. One also easily derives from (16) and (18)

$$L(Q, R) - L_1(Q, R) = \int_{C_1}[\text{grad } K_1(P, Q)\cdot \text{grad } L_1(P, R) +$$
$$q(P)K_1(P, Q)L_1(P, R)]\delta\nu ds + O(\epsilon^2). \quad (20)$$

These formulas are equivalent to the variational formulas for Green's and Neumann's functions which were given first by Hadamard in the case of Laplace's equation [2]:

$$\delta G(Q, R) = - \int_C \frac{\partial G(P, Q)}{\partial \nu_P} \frac{\partial G(P, R)}{\partial \nu_P} \delta\nu_P ds_P \quad (21)$$

$$\delta N(Q, R) = \int_C[\text{grad}_P N(P, Q)\cdot \text{grad}_P N(P, R) +$$
$$q(P)N(P, Q)N(P, R)]\delta\nu_P ds_P. \quad (21')$$

The significance of the series development (16) now becomes evident; it is a development of the functional $K(Q, R)$ of the domain D in powers of the Fréchet distance from the known domain D_1. The variational formulas (19–21') stand to this series in the same relation as do the expressions for differentials of functions of finitely many variables to the Taylor series expansions.

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MULTIPLICATIVE TRANSFORMATIONS

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Distributive operations over a linear space have been studied intensively in recent years. There has been comparatively little attention paid to the closely correlated considerations attached to multiplicative transformations. We shall be concerned throughout with the normed rings of real-valued continuous functions over compact sets. Perhaps the most interesting of our results for application in other fields are in connection with our definition of *vital* sets and Theorems 2 and 3. This last theorem seems unexpected for with no denumerability restrictions on neighborhood bases it asserts the maps of elements of our normed ring by a multiplicative functional are completely determined by the element values on a fixed closed countable set. Other topics cover deviations from strict multiplicativity and their consequences.

We use *countable* to cover either finite or denumerable. The complement of a set Q is written $\bar{Q}$. $C(S)$ is the usual normed ring of the real-valued continuous functions over compact S. We write $C_0(S)$ for the multiplicative semigroup of non-negative elements of $C(S)$ and $C_+(S)$ for the multiplicative group of positive elements. We designate the ring of real numbers by R whence the notations R_0 and R_+ are clear. The elements of $C(S)$ are denoted by small letters. Under the single-valued transformation T on $C(S_1)$ to $C(S_2)$ we shall use the capitalization of the small letter to indicate the correspondent. Thus $Tx = X$. We employ s and t for the points of S_1 and of S_2. Write $\Delta(xy)$ for $\|Txy - Tx\,Ty\|$ and $|x|_t$ for $\min_{S_1} |x(s)|$. If $|x|_t > 0$, x is regular. We indicate various types of non-multiplicativity [cf. reference 1] by saying T satisfies (A) or (B) or (C) where (A): $\Delta(xy) \leq \delta|x|_t\,|y|_t$, $\delta > 0$ (B) $\Delta(xy) = 0$ for $|xy|_t = 0$ and (C) $\Delta(xy) \leq \delta|xy|_t + \epsilon$.

The letters G and F with or without scripts invariably designate open and closed sets, respectively. If $\phi \neq F \subset G$ then since our compact S must be normal, functions $x(s)$ in $C(S)$ exist with $x|F = a$, $x|\bar{G} = b > a \geq 0$ and $a \leq x(s) \leq b$. We use the notation $U(F, G; a, b)$ for one of these functions chosen arbitrarily. For $(a, b) = (a, 1)$ and $(0, 1)$, respectively, we write $U(F, G; a)$ and $U(F, G)$.

Theorem 1. *If T satisfies (A) and S_1 has at least two points then T is actually multiplicative.*

If the assertion were untrue then for some x and y specified by primes, $(T(x'y') - Tx'\,Ty')(t) = D(t)$ does not vanish for $t \in Q$. Let $x_N(s) = U(F, G: N^{-1}, N)$ and let $y_N(s)$ be the reciprocal of $x_N(s)$. Observe

$$\|Tx_N(Tx'\ Ty' - Tx'y')\| \leq \|\ Ty'(Tx_NTx' - Tx_Nx')\ \| + \Delta((x_Nx')y') +$$

$$\Delta((x'y')x_N) \leq \frac{\delta}{N}\ [|x'|_t\|Ty'\| + |x'y'|_t + |y'|_t|x'|_t]. \quad (1.0)$$

Therefore, wherever $D(t) \neq 0$,

$$0 \leq |(Tx_N)(t)| \leq \alpha/N|D(t)|, \qquad \alpha \geq 0. \quad (1.01)$$

We have need of the following inequalities, valid when $t \in Q$.

$$\|(Tx'y' - Tx_Nx')Ty_Ny'\| \leq a/N^2,$$

$$\|(Tx'x_N - Tx'Tx_N)Ty'Ty_N\| \leq \|Ty'(Tx'x_NTy_N - Tx')\| \leq b/N^2,$$

$$|(Tx'x_N(Ty_Ny' - Ty'Ty_N))(t)| \leq |(Tx'x_N)(t)|\ |y'|_t\ \delta/N \leq c/N^2,$$

where the last is established by replacing x_N in (1) and (1.01) by (x_Nx'). Thus $|D(t)| \leq (a + b + c)/N^2$ in Q. That is to say $D(t) = 0$ everywhere, in contradiction with the assumed non-multiplicativity of T for the choice x', y'. An example[1] shows the theorem may be invalid if S_1 has just one point.

Let $\mathfrak{F}$ be a field of sets containing all closed sets (though similar concepts have merit with a less specialized $\mathfrak{F}$). Let P and P' designate mutually inconsistent set properties. We require: (1) If the sets A and B are in $\mathfrak{F}$ and $A \subset B$ then B has P' implies A has P'. (2) If A and B have P' then $A \cup B$ has P'. The set A is *vital* (with respect to P, P' and $\mathfrak{F}$) if $A \in \mathfrak{F}$ and if for every partition of A into disjunct sets B_1 and B_2 of $\mathfrak{F}$, B_i has P and $B_{i+1 \bmod 2}$ has P'.

THEOREM 2. *If (a) the null set and single points have P', (b) every set which has P has a compact subset which has P and (c) S is compact, then S contains no vital sets.*

Suppose to the contrary A is vital. If C_1 and C_2 are subsets of A in $\mathfrak{F}$ which have P then $C_1 \cap C_2$ has P. Otherwise $C_1 \cup (C_1 \cap C_2)$ would have P' by (2) and so C_2 would have P' by (1). By induction, if the sets C_i of $\mathfrak{F}$ have P then $\cap_i^{\ i} C_i$ has P whence according to (a) the closed sets in A which have P have F. I. P. (finite intersection property). Let $\{Q_\alpha\}$ be the totality of subsets of A in $\mathfrak{F}$ which have P and let $\{F_\beta\}$ be the sub-collection of closed sets in $\{Q_\alpha\}$. Then by virtue of (b) and (c) and the F. I. P. for $\{F_\beta\}$, $\cap F_\beta \supset \cap Q_\alpha \supset \cap F_\beta \Rightarrow \cap Q_\alpha = \cap F_\beta = R \neq \phi$. Suppose $x_0 \in R$. Then the dissection x_0, $A - x_0$ implies by (a) that $A - x_0$ has P whence $A - x_0 \in \{Q_\alpha\}$ and so $x_0 \bar{\in} R$ which is absurd. Theorem 2 is still valid for a locally compact S.

We shall call a completely additive, real-valued set function $\mu(Q)$ defined on the Borel sets of S, a B measure and we shall say μ is *continuous* if $\mu(Q) = 0$ for all countable sets Q. Suppose P stands for $\mu(A) = \delta > 0$ and P' for $\mu(A) = 0$. Take $\mathfrak{F}$ as the Borel field. Then A is vital if for

all dissections of A into disjunct Borel sets B_1 and B_2, $\mu(B_i) = 0$, $\mu(B_{i+1 \bmod 2}) = \mu(A) = \delta$. (The term "atom" has been used for a somewhat less restricted situation.)

$M(x)$ is a continuous multiplicative functional on $C(S)$ to R if $M(xy) = Mx\, My$ and $\|x^n - x\| \to 0$ implies $M(x^n) \to Mx$. We tacitly *bar* $M(x) = 0$ *or* $M(x) = 1$ *in the sequel*.

THEOREM 3. *If $M(x)$ is a continuous multiplicative functional on $C(S)$ to R, S compact, then there exists a countable closed set D in S independent of x such that $M(x)$ is completely determined by the values of $x(s)$ on D.*

We remark first that on the one hand $M|x|$ is non-negative since $|x|$ is a square and on the other $(M|x|)^2 = M(x^2) = (Mx)^2$ whence $|M(x)| = M|x|$. Hence we can consider Mx for x restricted to $C_0(S)$ in place of $|M(x)|$. Now M is a continuous multiplicative functional on $C_0(S)$ to R_0 and *a fortiori* on $C_+(S)$ to R_+. We sometimes indicate the domain of M by writing M_0 and M_+, respectively. We may consider M_0 as an extension of M_+. This simple observation actually points the way to the proof of our theorem.

If $x \in C_+(S)$ then $x(s) = \exp x'(s)$, where $x'(s) = \log x(s)$. We define the additive functional F by

$$F(x') = \log M(e^{x'}). \qquad (3.0)$$

In $C_+(S_1)$, $\|x^n - x\| \to 0$ implies $\|x'^n - x'\| \to 0$.

Let e be the function identically 1 on S. Then $M(x) = M(e)\, M(x)$ for all x whence $M(e) = 1$. Therefore $M(x^{-1}) = (M(x))^{-1}$ for regular x. For any integer k and $x \in C(S)$, we have $M(x^k) = (M(x))^k$ whence using $y = x^k$, $(M(y)^{1/k}) = (M(y))^{1/k}$. Accordingly $(M(y)^{1/k}) = (M(y))^{1/k}$. In short $(M(x)^r) = (M(x))^r$ for r rational but not necessarily positive. If $r \to \gamma$, then evidently $\|x^r - x^\gamma\| \to 0$ and so, for any real number γ,

$$M_+(x^\gamma) = (M_+(x)^\gamma). \qquad (3.01)$$

These facts show that F is a linear functional on $C(S)$. Therefore $F(x')$ has the representation,[2]

$$F(x') = \int_S x'(s)\, d\mu,$$

where μ is a *regular* B measure with $\mu(S) < \infty$, uniquely determined by F.

$$M_+(x) = \exp \int_S \log x(s)\, d\mu. \qquad (3.02)$$

The representation (3.02) is applicable to M_0 if we observe that M_0 is continuous so $M_+(x + \epsilon_n) \to M_0(x)$ for $\epsilon_n \downarrow 0$. Thus if $\int_S \log x(s)\, d\mu$ becomes negatively infinite it is merely necessary to set $M_0(x) = 0$.

Our key assertion is that the B measure μ is concentrated on a closed countable point set D. We assume the assertion false and derive a contradiction.

First we remark that the measure $\mu = \mu_1 - \mu_2$, where μ_1 and μ_2 are non-negative regular B measures. The continuity restriction on $M_0(x)$ requires that $M_0(x)$ be bounded. If $\mu_2 \neq 0$ then for some set A, $\mu(A) \leq -\delta < 0$. We have then by the regularity of the measure that a closed set F and an open set G exist with $F \subset A \subset G$ and $|\mu(B_1) - \mu(B_2)| < \delta/2$ for any two Borel sets boxed between F and G. Let $x_0 = U(F, G; \epsilon)$. Let $\{\lambda_n\}$ be a monotone increasing positive sequence with limit 1. Let $x_n = |x_0 - \lambda_n \epsilon|/1 - \lambda_n \epsilon$. Then $\int \log x_n \, d\mu \geq -(\log 2\epsilon + \log (1 - \lambda_n))\delta/2$ for $\bar{n} \geq N$, or $M(x_n) \geq (2\epsilon(1 - \lambda_n))^{-\delta/2}$. Plainly the sequence x_n converges but $M(x_n)$ does not. Accordingly $\mu_2 = 0$ and the B measure μ is actually *non-negative*.

Next let $D = \{s_i\}$ be the set of points such that $\mu(s_i) > 0$. Evidently D is countable. Let ν be the completely additive, non-negative regular set function on the Borel sets of S, defined by

$$\nu(A) = \mu(A) - \sum\mu(s_i|s_i \epsilon D \cap \dot{A}). \qquad (3.03)$$

Then there are no ν vital sets for in Theorem 2 (a) is obviously satisfied since $\nu(A)$ is continuous and the regularity of ν shows (b) is also. This sharpens the Kakutani representation[2] for linear functionals in $C(S)$. We digress to remark that for a *locally compact space* Theorem 2 implies the non existence of a two valved regular completely additive Borel measure vanishing on points if the measure and regularity are based in the usual way on compact subsets. (If however F and the notion of regularity are based on the closed sets such a measure can exist and Oxtoby has communicated an example to the writer).

Consider a sequence of positive numbers $\{a_n\}$ such that $\sum a_n < \infty$ and write Π_i^n for $(\Pi_{j=i}^n (1 + a_j))^{-1}$ subject to $\Pi_i^n = 0$ for $i < 0$, $\Pi_i^n = 1$ for $i > n$ and $\Pi_i = \Pi_i^\infty$. Define $(1 + i)^2 d_i$ as $1 - (\Pi_{i+1})$.

Admit $\nu(S)$ is positive. Suppose $\mu(G \cap D) = a$, $\nu(G) = b > 0$. Take an arbitrary positive integer m. Write $D^n = \{s_i|i \geq n\}$, $E^n = D - D^n$. For $n \geq N$ plainly $\mu(D^n) < a/2m$. Let $G_0 = G - E^n \cap G$. Since ν is continuous and admits no ν vital sets, G_0 can be partitioned into $m + 1$ disjunct Borel sets of positive ν measure whence one, say B, satisfies $0 < \nu(B) \leq b/n + 1$. In view of the regularity of ν some G_{00} and G' exist with $B \subset G_{00} \subset G_0$ and $G' \subset \bar{G}' \subset G_{00}$ with $0 < \nu(G') < \nu(G_{00}) < b/m$. Hence $\mu(G') \leq \nu(G') + \mu(G_0 \cap D) \leq (a + b)/m = \mu(G)/m$. Accordingly we can determine a sequence

$$G_0 \supset \bar{G}_1 \supset G_1 \supset \ldots \supset \bar{G}_i \supset G_i \supset \ldots \qquad (3.04)$$

with

$$\mu(\bar{G}_k) < d_k \mu(G_{k-1}). \qquad (3.05)$$

Consider the sequence defined by induction $x^1(s) = U(\bar{G}_1, G_0)$, ...
$x^{n+1}(s) = (a_n + x^n(s))(1 + a_n)^{-1} U(\bar{G}_{n+1}, G_n), \ldots$ For $s \, \epsilon \, \bar{G}_{i-1} \cap \bar{G}_i$

$$1 - \Pi_i^* \leq x^{n+1}(s) \leq 1 - \Pi_{i-1}^*. \tag{3.06}$$

If $n > m$, $x^{n+1}(s) - x^{m+1}(s) \leq (1 - \Pi_m)$. Accordingly x^n converges uniformly to a function $s \, \epsilon \, C_0(S)$. Since for fixed s $\{x^n(s)\}$ is monotone nondecreasing the zeros of $s(s)$ are contained in $\bigcap_{i-1} G_i$ and therefore in $\bigcap_{i-1} \bar{G}_i$. Clearly $\mu(\bigcap \bar{G}_i) \leq \mu(\bar{G}_n)$ for all n and so $\mu(\bigcap \bar{G}_i) = 0$. From (3.06) and $|\log a| < a^{-1}$ when $0 < a$, $|\int \log s(s) \, d\mu| < \sum(1 - \Pi_i)^{-1}$ $\mu(\bar{G}_{i-1}) \leq \sum_{i-1} i^{-2}$. In short $M(s) \neq 0$. However, since x^n vanishes on a set of positive measure $0 = Mx^n \to M(s)$. The contradiction establishes that $\nu(S) = 0$.

We show D is closed. Suppose s_0 is a cluster point of D with $s_0 \, \bar{\epsilon} \, D$, $\mu(s_0) > 0$. This implies the existence of x in $C_0(S)$ with $x(s_0) = 0$ and $M(x) > 0$. To show this observe that since $\{\mu(s_i)|s_i \, \epsilon \, D\} \, \epsilon \, h$ it is easy to demonstrate that for some strictly monotone increasing unbounded positive sequence $\{\lambda_n\}$, $\{\lambda_i \mu(s_i)\} \, \epsilon \, h$. Let $y(s) = \sum U(s_0, \, s_n)/2^n$. Then $y \, \epsilon \, C_0(S)$, $y(s_0) = 0$ and $2^{-i} \leq y(s_i) \leq 1$. Let h define a 1-1 order preserving correspondence between the denumerable closed subsets $0 \cup \{2^{-n}|n = 0, 1, \ldots\}$ and $0 \cup \{\exp - \lambda_n\}$ of the unit interval with $h(0) = 0$, $h(2^{-n}) = \exp - \lambda_n$. Then plainly h extends to an order preserving homeomorphism H of the unit interval into itself. Define $x(s)$ as $Hy(s)$. Note $x(s_0) = 0$ and $\exp - \lambda_i \leq x(s_i) \leq 1$. Accordingly $M(x) \geq \exp - \sum \mu(s_i)\lambda_i > 0$. Let $V_n = \{s|x(s) < \epsilon_n\}$, $W_n = \{s|x(s) \leq \epsilon_n/2\}$, where $0 < \epsilon_{n+1} < \epsilon_n/2$. Let $x^n(s) = U(W_n, V_n)x(s)$. Plainly $D \cap W_n \neq \phi$ and therefore $Mx^n = 0$. We next note $|x^n(s) - x(s)| < \epsilon_n$. Hence $x^n \to x$ and so $Mx = 0$. This contradiction establishes that actually $s_0 \, \epsilon \, D$.

The representation of $|Mx|$ is then plainly

$$|M(x)| = \Pi |x(s_i)|^{\mu(s_i)}. \tag{3.07}$$

Since $\int \log x(s) \, d\mu$ is of course absolutely convergent the infinite product is absolutely convergent. Incidentally note D is not vacuous.

We finish our proof by showing sign $M(x)$ is determined by the values of x on D also. We need only disprove the possibility that $x_1(s) = x_2(s)$ on D but sign $M(x_1) = 1$, sign $Mx_2 = -1$. If $z_\lambda = \lambda x_1 + (1 - \lambda)x_2, 0 \leq \lambda \leq 1$, $M(z_\lambda)$ is continuous in λ and since $|M(z_\lambda)| = |M(x_i)|$, $i = 1, 2$, sign Mz_λ must vary continuously from -1 to 1 which is absurd since the sign ( ) function can assume only 3 values.

Accordingly, taking into account also the lemma below, sign $M(x)$ is 0 for non-regular x or is given by any continuous homomorphism, h (sign $x(D)$), of the multiplicative group of the obvious equivalence classes (according to sign) of the regular elements of $C(D)$, D a denumerable

compactum, to the multiplicative group with two elements $(1, -1)$. A natural designation for D is therefore that of "determining set." We remark that if the range of M is K the complex number field then the determining set D is again a denumerable compactum. Indeed (3.07) retains its validity for $M|x|$ provided $\mu(s|D)$ is replaced by $\mu_1(s|D) + i\mu_2(s|D)$ where $\mu_1(s_i)$ is positive but $\mu_2(s_i)$ may be of either sign or 0.

LEMMA. *A denumerable closed subset D of a compact (Hausdorff) set S is a compactum in the induced topology.*

We remark that in view of well-known theorems it is sufficient to demonstrate a countable neighborhood basis in D at each point s of D. If $\tilde{s}$ is isolated, then, for some neighborhood U in S, $U\tilde{s} \cap D - \tilde{s} = \phi$ and $\tilde{s}$ is itself a neighborhood. If s is not isolated then for each s distinct from $\tilde{s}$ there is a neighborhood of $\tilde{s}$ in S, which does not contain s. Denote the totality of these neighborhoods by $\{U^i|i = 1, 2, \ldots\}$. Choose the neighborhoods V^i so that $\tilde{s} \subset V^i \subset \bar{v}^i \subset U^i$ and let $W^i = \bigcap_{j=1}^{i} V^j$. The corresponding neighborhoods in D are denoted by the corresponding small letters. Thus $w^i = w^i \cap D$, $v^i = V^i \cap D$, etc. We assert $\{w_i\}$ is a neighborhood basis at $\tilde{s}$. Otherwise for some neighborhood of $\tilde{s}$, say g, $w^i \cap \tilde{g} \neq 0$ for all i. Select $s^i \in w^i \cap \tilde{g}$. Compactness guarantees a cluster point $s \in D$. However $s \bar{\in} g$ since s^i is foreign to g. Thus $s \neq \tilde{s}$ and since $\tilde{s} = \bigcap u^i \supset \bigcap \bar{v}^i$ we infer $s \bar{\in} \bar{v}^i$ for some i. Therefore some neighborhood of s includes no s^j for $j \geq i$ in contradiction with the fact that s is a cluster point.

If $x \in C(S)$ then $x|D \in C(D)$. Tietze's theorem shows every continuous function on D is extensible to a continuous function on S. It follows that the representation problem for sign $M(x)$ is reducible to the case of continuous functions on a countable compactum.

THEOREM 4. *If S_i is compact $i = 1, 2$, and if (a) T satisfies B and (b) at least one non-regular element of $C(S_1)$ is not mapped into the zero element of $C(S_2)$ then, if (c) all of $C(S_1)$ is covered, T is multiplicative.*

Let $Q = \{t|(Tx)(t) \neq 0$ for some non-regular $x \in C(S_1)\}$. It is known [Lemma 7, reference 1] that $(Tx)(t)$ is multiplicative for $t \in Q$. (This establishes the theorem if S_2 has just one point.)

We contend that for $t_1 \in Q$, $(Tx)(t_1) = 0$ implies x is non-regular. Otherwise x^{-1} exists and for any $s \in C(S_1)$, $(Ts)(t_1) = (Txx^{-1}s)(t_1) = (TxTx^{-1}s)(t_1) = 0$. This denies (c).

Suppose T is not multiplicative then $\tilde{Q}$ contains a point designated by t_0. Let $t_1 \in Q$. Consider $Y \in C(S_2)$ with $Y(t_1) = 0$ and $Y(t_0) \neq 0$. Suppose $Y = Ty$. Obviously y is regular since $t_0 \in \tilde{Q}$. On the other hand $(Ty)(t_1) = 0$ requires y be non-regular. The contradiction establishes the theorem. Examples[1] show that neither (b) nor (c) can be dispensed with. Observe (a), (b), (c) are not consistent if S has just one point.

We now consider relation (C). (The parenthetical remark on page 392

of reference 1 referring to the connection between the relation referred to in that paper by A and our present C is not quite correct without further restriction.)

LEMMA. *If T satisfies (C) and for every $t_1 \epsilon S_1$ there exists a sequence of functions w_N in $C(S_1)$ possibly dependent on the choice of t_1 such that $(Tw_N)(t_1) \to \infty$ and if $|xy|_t = 0$ then T is multiplicative on xy.*

Suppose $|y|_t = 0$ then

$$|((Txy - Tx\,Ty)Tw_N)(t_1)| \leq \Delta((xy)w_N) + \Delta(x(yw_N)) + \|Tx(Tw_N - TyTw_N)\| \leq \epsilon(2 + \|Tx\|).$$

In short $|(Txy - Tx\,Ty)(t_1)| \leq \epsilon(2 + \|Tx\|)/|(Tw_N)(t_1)| \to 0$.

THEOREM 5. *If T satisfies C and is $1 - 1$ on $C(S_1)$ onto $C(S_2)$ then S_1 and S_2 are homeomorphic.*

Indeed since the mapping is "onto" the sequence w_N exists for every choice of t_1 in S_2 and *hence $|xy|_t = 0$ implies $Txy = Tx\,Ty$.* Theorem 4 now guarantees T is actually multiplicative and our result follows from Theorem 6 of reference 1 or from reference 3.

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EXTENSIVE GAMES*

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In the mathematical theory of games of strategy as described by von Neumann and Morgenstern,[1] the development proceeds in two main steps: (1) the presentation of an all-inclusive formal characterization of a general n-person game, (2) the introduction of the concept of a pure strategy which makes possible a radical simplification of this scheme, replacing an arbitrary game by a suitable prototype game. These two forms have been given technical names by von Neumann and Morgenstern, who called them the *extensive* and the *normalized* forms of a game. As they have noted, the normalized form is better suited to the derivation of

general theorems (e.g., the main theorem of the zero-sum two-person game), while the extensive form exposes the characteristic differences between games and the decisive structural features which determine those differences. Since all games are found in extensive form, while it is practical to normalize but a few, it seems reasonable to attack the completion of a general theory of games in extensive form. This note presents two new results in this theory which appear to have far-reaching consequences in the computational problems of normalized games. These results are cast in terms of a new formulation of the extensive form which seems to have intuitive advantages over that used by von Neumann.[2]

In order to engage in a precise discussion, it will be necessary to clarify certain concepts associated with a game which are confused and ambiguous in common use. We will use these terms in essentially the same manner as von Neumann and Morgenstern. A *game* is simply the set of rules which define it, while every particular instance in which a game is played from beginning to end is called a *play* of that game. A similar distinction is drawn between the occasion of the selection of one among several alternatives, to be made by one of the players or by some chance device, which is called a *move* and the actual selection in a particular play which is called a *choice*. Thus, a game consists of a set of moves in some order (not necessarily linear!), while a play consists of a sequence of choices.

1. The Extensive Form.—

Definition: A general n-person game Γ is a finite tree K imbedded in an oriented plane with the following specifications:

(1) A distinguished vertex O.

(*Terminology:* The *alternatives* at a vertex P are the edges ϵ incident to P and lying in components not containing O if we cut K at P. If there are j alternatives at P, then we index these by the integers $1, \ldots, j$, circling P in the positive sense. At the vertex O, the first alternative may be assigned arbitrarily. If we circle a vertex $P \neq O$ in the positive sense, the first alternative follows the unique edge at P which is not an alternative. Those vertices which possess alternatives will be called *moves;* the remaining vertices will be called *plays*. We define a partition[3] of the moves into sets $A_j, j = 1, 2, \ldots$, where A_j contains all of the moves with j alternatives, which will be called the *alternative partition*.

We also introduce a *temporal order* in the tree K. As in any tree with base point O, there is a uniquely defined unicursal path W_P leading from O to the vertex P. We say that $P \leq Q$ whenever $P \epsilon W_Q$. This clearly defines a partial ordering of the vertices of K and enables us to assign a (temporal) *rank* to the vertices as follows: O is of rank 1. A vertex P is of rank k if the maximum of the rank of Q such that $Q < P$ is $k - 1$. Using this definition we can introduce the *rank partition* of the moves into sets M_k consisting of all moves of rank k for $k = 1, 2, \ldots$.)

(2)   A partition of the moves into $n + 1$ indexed sets $P_0, P_1, \ldots, P_n$ which will be called the *player partition*.

(*Terminology:* The moves lying in P_0 are called *chance moves;* all other moves are called *personal moves.*)

(3)   A partition of the moves into sets U which is a refinement of the alternative, player and rank partitions, that is, each U is contained in $P_i \cap A_j \cap M_k$ for some i, j and k.   This partition is called the *information partition* and its sets will be called *information sets.*

(4)   For each $U \subset P_0 \cap A_j$, a probability distribution on the integers $1, \ldots, j$, which assigns *positive* probability to each.   Such U are assumed to be one-element sets.

(5)   An n-tuple of real numbers $h(W) = (h_1(W), \ldots, h_n(W))$ for each play W.

(*Terminology:* The function h specified in (5) is called the *pay-off function.*)

The question which must be answered immediately is: How is this formal scheme to be interpreted?   That is, how does one play our general n-person game Γ?   To personalize the interpretation, one may imagine a number of people isolated from each other with contact with a single person, termed the *umpire*.   All persons involved are supposed to know the rules of the game; that is, each is to have a copy of the tree K and the specifications (1)–(5).   We assume that there is one person for each information set and that they are grouped into players in the natural manner, a person belonging to the ith player if his information set lies in P_i.   This seeming plethora of persons is occasioned by the possibly complicated state of information of our players who may be called upon to forget facts which they knew earlier in a play.[4]

A play begins at the vertex O.   We do not exclude the possibility that this is the only vertex in K; then we have a no-move game, no one does anything and the pay-off is $h(O) = (h_1(O), \ldots, h_n(O))$.   Suppose that the play has progressed to the move P.   Then the play continues by the umpire contacting the person whose information set contains P and, if P is a personal move with j alternatives, asking him to choose a positive integer not greater than j.   The person does this, knowing only that he is choosing an alternative at one of the moves in his information set.   We assume that the umpire makes all of the chance choices in advance, in accord with the probabilities assigned in (4), so that if P is a chance move then an alternative has already been chosen.   In this manner, a path with initial point O is constructed.   It is unicursal and hence leads to a play W.   (Henceforth we shall utilize the 1–1 correspondence between the plays, which are vertices, and the unicursal paths from O to the plays and use the name play for both objects when no confusion will result.)   At this point, the umpire pays player i the amount $h_i(W)$ for $i = 1, \ldots, n$.

A detailed comparison of our formal scheme and von Neumann's axiomatic formulation[5] reveals that if we derive one of our games from a von Neumann game in a natural way the only condition imposed is that all of the plays be of the same rank. This is essentially trivial and can be satisfied by filling out "short" plays with dummy chance moves with only one alternative. Proceeding in the converse direction, if we take one of our games in which all of the plays have the same rank, we impose the single restriction that all of the plays in Ω be admissible under the rules of Γ. Thus we have axiomatized essentially the same set of objects as von Neumann.

2. *Pure, Mixed and Behavior Strategies.*—Rather than make each decision separately as the occasion demands, a player may devise a plan in advance to cover all possible situations which may confront him. He loses nothing by doing this since he makes his choice a function of the information available to him; consequently, his choice must be constant over each of his information sets. Such a plan is called a *pure strategy.*

Definition: A *pure strategy* π_i for player i in Γ is a choice of a positive integer not greater than j for each set $U \subset P_i \cap A_j$.

If the players choose pure strategies $\pi_1, \ldots, \pi_n$ then a probability p_e is assigned to each alternative e in the graph K; if e is a chance alternative, then p_e is obtained from specification (4), while if e is the νth alternative at a personal move in P_i then $p_e = 1$ if π_i specifies the choice ν on the set U containing this move and $p_e = 0$ otherwise. Clearly, the probability $p_W(\pi_1, \ldots, \pi_n)$ that a play W will occur is given by the formula:

$$p_W(\pi_1, \ldots, \pi_n) = \prod_{e \, \epsilon \, W} p_e(\pi_1, \ldots, \pi_n), \qquad (6)$$

and hence the expected pay-off to player i is given by

$$H_i(\pi_1, \ldots, \pi_n) = \sum_W p_W(\pi_1, \ldots, \pi_n) h_i(W). \qquad (7)$$

Unfortunately, our definition of a pure strategy, while conceptually simple, has an inherent redundancy which we will now eliminate. This redundancy is simple in nature; in the case of a zero-sum two-person game, it is merely the duplication of rows and columns in the pay-off matrix.

Definition: We shall say that two pure strategies for player i are *equivalent*, written $\pi_i \equiv \pi_i'$, if and only if $p_W(\pi_1, \ldots, \pi_i, \ldots, \pi_n) = p_W(\pi_1, \ldots, \pi_i', \ldots, \pi_n)$ for all plays W and all pure strategies for the remaining players, $\pi_1, \ldots, \pi_{i-1}, \pi_{i+1}, \ldots, \pi_n$.

The following definition provides the working criterion for the equivalence of pure strategies.

Definition: A personal move P for player i is called *possible when playing* π_i if there exists a play W and pure strategies for the remaining players $\pi_1, \ldots, \pi_{i-1}, \pi_{i+1}, \ldots, \pi_n$ such that $p_W(\pi_1, \ldots, \pi_i, \ldots, \pi_n) > 0$ and $P \in W$.

Criterion: The pure strategies π_i and π_i' are equivalent if and only if they have the same set of possible moves and specify the same choices on those moves.

Henceforth, when we speak of a pure strategy, we shall mean an equivalence class under the definition just given. Clearly, formulae (6) and (7) still hold, where if e is an alternative specified by a pure strategy' on an information set containing a possible move then $p_e = 1$ and $p_e = 0$ otherwise.

However, the simplest games (e.g., Matching Pennies) reveal that a player is at a disadvantage if he uses the same pure strategy in each play. Instead, he can use a probability distribution μ_i on his pure strategies which we will call a *mixed strategy.* Now let π_i appear in the mixed strategy μ_i with probability q_{π_i}. Then the probability that a given play W will result is:

$$p_W(\mu_1, \ldots, \mu_n) = \sum_{\pi_1, \ldots, \pi_n} q_{\pi_1} \cdots q_{\pi_n} p_W(\pi_1, \ldots, \pi_n) \qquad (8)$$

and the expected pay-off to player i is given by

$$H_i(\mu_1, \ldots, \mu_n) = \sum_W p_W(\mu_1, \ldots, \mu_n) h_i(W)$$

$$= \sum_{\pi_1, \ldots, \pi_n} q_{\pi_1} \cdots q_{\pi_n} H_i(\pi_1, \ldots, \pi_n). \qquad (9)$$

In solving games in normalized form it has been customary to deal with the mixed strategies just introduced. However, instead of mixing pure strategies, a player could specify a probability distribution over the alternatives in each information set and thus plan his action in any given play. We will call the aggregate of such distributions a *behavior strategy.* The advantage of dealing with behavior strategies is a radical reduction of the dimension of the sets involved while the obvious disadvantage derives from a loss of freedom of action. Behavior strategies have been used with telling effect in the solution of individual games by von Neumann,[6] J. Nash and L. S. Shapley,[7] and the author.[8] In our formal treatment we will only deal with behavior strategies which are derived from a mixed strategy.

Definition: Suppose that the mixed strategy μ_i assigns the probability q_{π_i} to each pure strategy π_i and consider the information set U for player i with j alternatives. Let S be the set of pure strategies π_i such that some $P \in U$ is possible when playing π_i. Then S is the disjoint union of the sets $S_1, \ldots, S_j$ where S_ν consists of all π_i which specify the νth alternative on U. If $\sum_{\pi_i \in S} q_{\pi_i} \neq 0$ then we define:

$$b_\nu = \sum_{\pi_i \epsilon S_\nu} q_{\pi_i} \Big/ \sum_{\pi_i \epsilon S} q_{\pi_i} \quad \text{for } \nu = 1, \ldots, j. \tag{10}$$

If $\sum_{\pi_i \epsilon S} q_{\pi_i} = 0$ then no distribution is defined. The aggregate of all such assignments on information sets U for player i is called the *behavior strategy associated with* μ_i and is denoted by $\beta_i(\mu_i)$ or simply β_i.

Again, if the players choose behavior strategies $\beta_1, \ldots, \beta_n$ then a probability p_e is assigned to each alternative e in the graph K; if e is a chance alternative, then p_e is obtained from specification (4), while if e is the νth alternative at a personal move in P_i at which β_i assigns the probability b_ν then $p_e = b_\nu$ and $p_e = 0$ otherwise. Clearly, the probability $p_W(\beta_1, \ldots, \beta_n)$ that a play W will occur is given by the formula:

$$p_W(\beta_1, \ldots, \beta_n) = \prod_{e \epsilon W} p_e(\beta_1, \ldots, \beta_n) \tag{11}$$

and hence the expected pay-off to player i is given by

$$H_i(\beta_1, \ldots, \beta_n) = \sum_W p_W(\beta_1, \ldots, \beta_n) h_i(W). \tag{12}$$

3. Games with Perfect Information and Games with Perfect Recall.—
We shall be concerned with two large classes of games in which the information partition assumes a special form.

Definition: A game Γ is said to have *perfect information*[9] if the information partition consists of one-element sets.

Definition: A game Γ is said to have *perfect recall for player i* if, for all pairs of moves P, Q for player i such that $P < Q$, we have the following condition satisfied. Assume that P and Q lie in the information sets U and V, respectively. Let P have j alternatives and let V_ν be the set of all moves following some $R \epsilon U$ in the temporal order in a play which has the νth alternative at R. Then we demand that $V \subset V_\nu$ for some ν. A game Γ is said to have *perfect recall* if it has such for all players.

The interpretation of these terms is exactly what the names imply. In a game with perfect information, each player is informed at every move of the exact sequence of choices preceding that move. In a game with perfect recall each player remembers everything that he knew and all of his choices at previous moves. The following two theorems hold for such games.

THEOREM 1. *A sufficient condition that an n-person game Γ have an equilibrium point*[10] *among the pure strategies for all possible assignments of the pay-off function h is that Γ have perfect information.*

THEOREM 2. *A necessary and sufficient condition that*

$$H_i(\beta_1(\mu_1), \ldots, \beta_n(\mu_n)) = H_i(\mu_1, \ldots, \mu_n) \tag{13}$$

for all mixed strategies $\mu_1, \ldots, \mu_n$ *and* $i = 1, \ldots, n$ *in an n-person game* Γ *for all possible assignments of the pay-off function h is that* Γ *have total recall.*

Theorem 1 generalizes the theorem of von Neumann which asserts that a zero-sum two-person game with perfect information is strictly determined. It is proved by the same inductive device with a slight variation due to the absence of the minorant and majorant games in the general n-person case. Theorem 2 enables us to replace mixed strategies by behavior strategies in games with total recall and has many computational ramifications. The proofs of both of the theorems and further considerations of extensive games will be published elsewhere.

* Written under a contract with the Office of Naval Research.

[1] Neumann, J. von, and Morgenstern, O., *The Theory of Games and Economic Behavior*, 2nd ed., Princeton University Press, 1947.

[2] A graphical representation by a tree has been suggested by von Neumann, *loc. cit.*, p. 77, however he does not treat this matter systematically, preferring a set theoretical formulation.

[3] In this paper a partition means an exhaustive decomposition into (possibly void) disjoint sets.

[4] It has been noted by von Neumann that Bridge is a two-player game in exactly this manner.

[5] Neumann, J. von, and Morgenstern, O., *loc. cit.*, pp. 87–84.

[6] Neumann, J. von, and Morgenstern, O., *loc. cit.*, pp. 192–194.

[7] Nash, J., and Shapley, L., "A Simple Three-Person Poker Game," *Annals of Mathematics*, Study No. 24 (in preparation).

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[10] Nash, J., "Equilibrium Points in n-Person Games," these PROCEEDINGS, **36**, 48–49 (1950).

THE SPECIFICITY OF ANTI-KIDNEY ANTIBODY DETERMINED BY ITS EFFECT UPON TISSUE CULTURE EXPLANTS

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While investigating the pathogenesis of experimental nephritis produced by rabbit anti-rat-kidney antibody, it occurred to us that the effects of anti-kidney antibody on kidney tissue might readily be visualized in tissue cultures. The specificity of tissue antigens has previously been investigated by the usual immunologic procedures[1] and the effects of antibodies[2,3] upon tissue explants has long been known. The growth and function of tissue explants have previously been used to study the specificity of tissue

antibodies,[4, 5] but the antisera used were of low, uncertain potency, and only a few different tissues were examined in testing the toxic effects. The following study shows that rabbit anti-rat-kidney gamma globulin is toxic for tissue explants of brain and heart muscle, as well as kidney. These effects are of low species specificity and may be observed in cultures prepared from chick embryo tissues, as well as those prepared from rat tissues. In addition, the serum of patients with glomerular nephritis was found to contain a substance with similar effects and specificity.

Methods.—Rabbit anti-rat-kidney serum was prepared by immunizing rabbits over a period of 4 months with a suspension of rat kidney tissue. The serum was first adsorbed with a suspension of sheep erythrocytes, to remove the Forssman antibodies, and then with rat erythrocytes. The antibody globulin was precipitated by $^1/_3$ saturation with ammonium sulfate at pH 7.8. This preparation (NTG) was found by the Tiselius pattern to be composed entirely of gamma globulin. The minimal serological activity was determined by a precipitin test with the soluble portion of the original antigen and the preparation NTG was found to contain at least 12% precipitable antibody.

Tissue explants were cultured by the usual double cover slip method, which has been described in detail.[6] In a five-drop portion of culture medium, one drop of NTG (32.5 mg. protein/ml.) was substituted for one drop of serum. Controls were concurrently grown in a medium that substituted one drop of gamma globulin prepared from unimmunized rabbit serum (GC) for one drop of serum. Controls were also grown, in most cases, in a medium of the usual formula without substitution (SC).

Cultures were examined at intervals up to 72 hours, when the experiments were usually terminated, and were graded for growth and evidence of functional activity. At least 4 explants were grown under each condition, and the key conditions were repeated for several groups of 4 explants each. The results were completely uniform. Photographs were taken of typical preparations for permanent record, and some of the tissues were then fixed and stained.

Species Specificity.—Explants of rat kidney in SC and GC grew very well. Those in NTG showed virtually no growth, and in the few cells that grew out from the explant the cytoplasm was granular, with fatty degeneration. Most of these cells were dying, and many became autolyzed during the period of observation.

In order to provide a crucial test of species specificity, explants of chick mesonephros were grown in rat NTG. Almost no growth occurred. In addition, tubular function in the explant, easily visualized by the incorporation of a low concentration of phenol red in the medium,[7] rapidly came to a halt. In contrast, excellent growth and function were observed in SC and GC preparations.

Organ Specificity.—In order to study organ specificity of NTG, tissue explants from different organs of the rat and of the chick embryo were grown in the various media. The results are shown in table 1. As seen from the table, growth of kidney, heart and brain explants was inhibited by NTG. No other tissues were affected, although a wide variety derived from all embryonic layers was examined.

Circulating Nephrotoxin in Patients with Glomerular Nephritis.—It has been well known, since the work of Masugi,[3] that a form of nephritis may

TABLE 1

EFFECT OF RABBIT ANTI-RAT-KIDNEY GLOBULIN ON TISSUE EXPLANTS FROM THE CHICK EMBRYO AND FROM THE RAT

SPECIES	TISSUE	SERUM CONTROL (sc)	GLOBULIN CONTROL (oc)	NEPHROTOXIC GLOBULIN (NTG)
Rat	Kidney	0	0	+
	Heart	0	0	+
	Brain	0	0	+
	Liver	*	0	0
	Bladder	*	0	0
	Spleen	*	0	0
Chick	Mesonephros	0	0	+
	Heart	0	0	+
	Brain	0	0	+
	Liver	*	0	0
	Striated muscle	0	0	0
	Lung	0	0	0
	Intestinal smooth muscle	0	0	0
	Intestinal mucosa	0	0	0
	Skin	0	0	0
	Tongue	0	0	0
	Chorio-allantoic membrane	*	0	0

+ = marked inhibition of growth, 0 = no effect on growth.

* No experiment performed.

be produced in animals by the administration of anti-kidney serum. Lange and his coworkers have reported the occurrence of "auto-antibodies" to kidney tissue in human nephritis.[9] For these reasons, it seemed desirable to test the serum of patients with glomerular nephritis, but without renal failure, for the presence of a circulating nephrotoxin. The serum of 6 patients and 4 normal individuals has been tested in this way, and the results are presented in table 2. Serum from all of the patients with well-documented glomerular nephritis completely inhibited the growth and tubular function of explants from the chick mesonephros. Growth of chick heart

and brain explants was also inhibited.   Serum from normal individuals had no effect upon the explants when compared with controls grown in SC.

Comments.—It is of considerable interest that rabbit anti-rat-kidney gamma globulin inhibits the growth in tissue culture of rat heart muscle as well as rat kidney, chick brain, chick heart muscle and chick mesonephros. The presence of a common antigen in these tissues would render reasonable the clinical association of such conditions as glomerular nephritis, rheumatic carditis and chorea.

Even more interesting is the observation that the serum of patients with glomerular nephritis contains a substance which is directly toxic to kidney, heart and brain tissue.   Since the patients were selected for adequate renal function, the effect probably cannot be attributed to accumulated waste metabolites in the serum.

TABLE 2

EFFECT ON GROWTH OF CHICK EMBRYO TISSUES OF SERA FROM NORMAL INDIVIDUALS AND FROM PATIENTS WITH GLOMERULAR NEPHRITIS

SUBJECT	DIAGNOSIS	STAGE[16]	MESO-NEPHROS	HEART	BRAIN	STRIATED MUSCLE
R. L.	Normal	...	0	0	0	0
H. U.	Normal	...	0	0	0	0
H. Y.	Normal	...	0	0	0	0
A. M.	Normal	...	0	0	0	0
B. M.	Glom. neph.	Initial	+	+	+	0
A. R.	Glom. neph.	Degenerative	+	+	+	0
K. R.	Glom. neph.	Degenerative	+	+	+	0
D. W.	Glom. neph.	Degenerative	+	+	+	0
C. B.	Glom. neph.	Degenerative	+	+	+	0
W. R.	Glom. neph.	Degenerative	+	+	+	0

+ = marked inhibition of growth, 0 = no effect on growth.

Further experiments to advance all phases of this study are in progress. It is hoped that we shall be able to characterize more completely the organ and species specificity of several tissue antigens and that we shall be able to study in more detail the localization of antibody activity in the various serum protein fractions.   Sera from the patients will be fractionated in order to identify the nephrotoxin.   It is also hoped that these studies will lead to a simple test for the presence of tissue toxins, and an effort will be made to neutralize such a substance *in vivo.*

The technique of tissue culture offers a sensitive and relatively simple way to examine sera for the presence of tissue toxins and antibodies.   It is our ultimate intention to extend these studies to patients with other diseases thought to be related to tissue allergies, such as lupus erythematosus disseminatus, rheumatic fever, rheumatoid arthritis and others.

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THE EXCITATORY PROCESS IN THE COCHLEA*

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Introduction.—The nature of the excitatory process by which sensory cells initiate nerve impulses in afferent nerve fibers is very obscure.   In the case of the ear, the most widely accepted theory has been that an electrical potential known as the "cochlear microphonic" is generated by the hair cells in the organ of Corti and serves as a direct electrical stimulus to the peripheral terminations of the fibers of the auditory nerve.

The cochlear microphonic, it will be recalled, seems to be simultaneous with the mechanical movements of the cochlear partition and it follows the wave form of the sound very closely, at least at moderate intensities of stimulation.   It shows no refractory period or all-or-none characteristics like the action potentials of nerve.[1]   Action potentials of the auditory nerve, probably generated in the cell bodies in the spiral ganglion, not in the fine non-myelinated terminal twigs, can also be recorded from electrodes placed in or near the cochlea.

Sound waves at 2000 to 6000 cycles per second are very efficient as auditory stimuli. This high efficiency led Wever[1] and one of the present authors[2] to postulate an intermediate excitatory effect or process which had the property of summating the excitatory effects of two or more sound waves. We now present experimental evidence for the existence of such a process of summation. We also have found a third electrical potential in the cochlea in addition to the cochlear microphonic and nerve action potentials. The new potential exhibits summation and seems to represent the local excitatory process that initiates auditory nerve impulses.

Methods.—All experiments were performed on guinea pigs anesthetized with dial in urethane. Electrodes consisting of No. 38 enamel-insulated silver wire were introduced into the cochlea through small holes drilled into scala tympani, scala vestibuli and/or scala media[3,4] of the basal turn.

ELECTRODES IN FIRST TURN OF GUINEA PIG'S COCHLEA
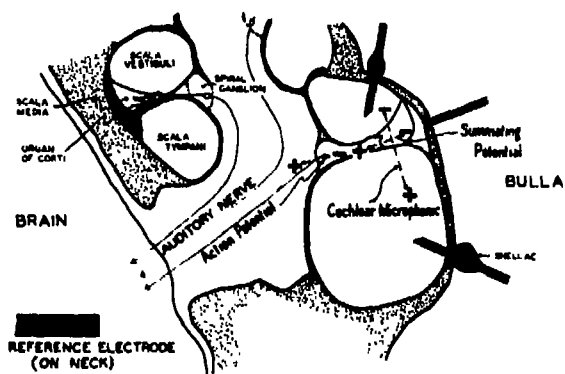
FIGURE 1

(In some experiments electrodes were placed in other turns as well.) The position in turn 1 is at the site of maximal stimulation by tones of 8000 c. p. s. The electrical circuit was completed by a reference electrode attached to the wound in the neck. The cochlea of the guinea pig protrudes into an air-filled bulla, and thus the electrical circuit from the reference electrode enters the cochlea chiefly by way of the internal auditory meatus. The reference electrode is thus roughly equivalent to one placed on the auditory nerve.

Electrical responses were recorded simultaneously from several combinations of electrodes by means of a three-channel cathode-ray oscilloscope assembly (Grass). Two of the channels could be connected in parallel so as to either add or subtract the signal in one to (or from) the signal in the other.[3,4] In this way the action potential of the auditory nerve, which appears as a negative electrical change at all of the cochlear electrodes,

could be completely canceled by subtracting the scala tympani signal from the scala vestibuli signal, after appropriate adjustment of amplification. The cochlear microphonic, on the other hand, appears in opposite phase in scala tympani from what it is in scala vestibuli and scala media (see Fig. 1). It can be canceled by adding the signals from scala vestibuli (or media) and scala tympani. By this method action potentials of moderate voltage can be viewed, and their latency measured, in spite of the simultaneous presence of a large cochlear microphonic which otherwise usually obscures them almost completely.

The acoustic stimuli employed included clicks and pure tones, but were chiefly brief tone-pips. Our usual "tone-pip" consisted of sound waves at a basic frequency of 8000 c. p. s. This signal begins gradually and reaches maximum amplitude during the fourth or fifth wave and immediately diminishes again (see Fig. 3). The "2000 c. p. s. tone-pip" has a basic frequency of 2000 c. p. s. The maximum in this case is reached
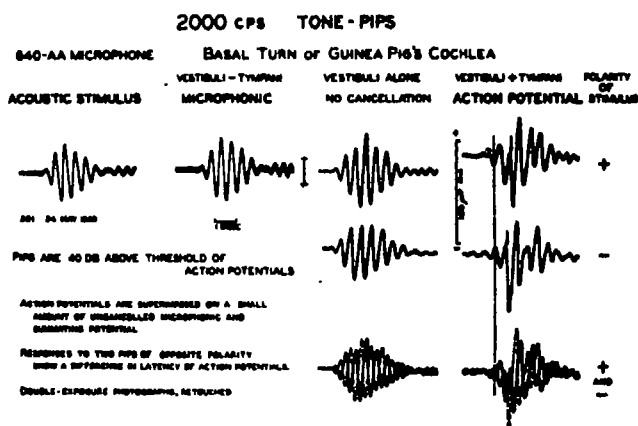
FIGURE 3

during the third sound wave. The pips were presented at pulsing frequencies from 1 to 60 per second. The tone-pips have several advantages: (1) nearly all of the acoustic energy is concentrated in a band less than an octave wide[5] so that they presumably activate a relatively restricted region of the cochlear partition, yet (2) the relatively rapid onset may allow identification of the particular sound wave that initiates a nerve impulse and (3) at frequencies of 1000 c. p. s. and higher only one impulse is set up in each fiber because of the rapid increment and decrement of sound waves and the refractory period of the nerve fibers.

Evidence for Summation.—For frequencies of 2000 c. p. s. and less, each volley of nerve impulses revealed by the action potential can be clearly associated with one sound wave or another. We find that only the portion

of the wave corresponding to acoustic rarefaction at the ear drum causes auditory stimulation. If one (negative) wave in a tone-pip has set up an action potential, the next wave, if stronger, may activate other neurons of higher threshold even though the neurons stimulated by the first wave are still refractory. Thus the composite action potential may show two peaks separated by one wave-length of the basic frequency of the tone-pip (see Fig. 2). Reversal of the polarity of the tone-pip, i.e., starting with a negative instead of a positive sound wave, will change the latency of the first effective sound wave because the first adequate negative sound wave now comes either a half wave earlier or a half wave later than it did previously. (The first effective wave may be either stronger or weaker than the first effective wave with the original polarity. The two peaks of action potentials will in general, therefore, be different in amplitude as well as showing a difference in latency. Both of these effects are illustrated in figure 2.) The basic frequency employed in figure 2 was 2000 c. p. s. and it is clear that at this frequency the auditory nerve is responding to individual sound waves in the tone-pip.

FIGURE 3
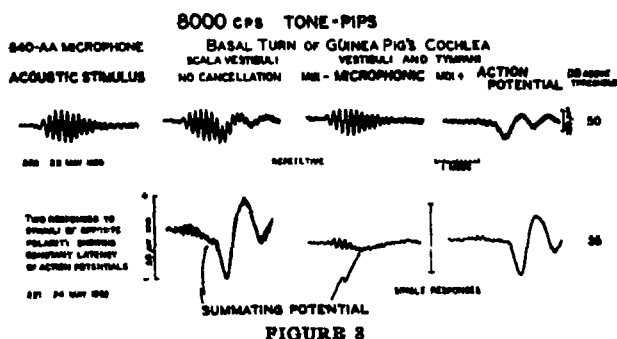
With a basic frequency of 8000 c. p. s., however, the behavior of the nerve is fundamentally different. There is no visible change in latency when the polarity of the tone-pip is reversed (see Fig. 3). We have sought for a change of latency with great care on the face of the oscilloscope and have assured ourselves that if there is a change of latency it is certainly less than 30 microseconds. The change which should occur if the nerve were responding to individual sound waves is 62 microseconds. We conclude that at this frequency the nerve does not respond to individual sound waves but that a stimulating effect is carried over, even from the earliest subliminal waves. The stimulating effects are integrated by some process that we call "summation" so that the nerve responds to the tone-pip as a whole instead of to the individual waves. Summation seems to dominate the picture at 8000 c. p. s. (At 4000 c. p. s. a small change of latency with reversal of polarity can still be demonstrated, however.)

A second line of evidence for summation at high frequencies is found in the change of latency with the intensity of a tone-pip. At low frequencies the latency diminishes in stepwise fashion as the intensity of the stimulus is increased. Each step means that an earlier sound wave, originally too weak to stimulate, has now become strong enough to set up nerve impulses. At 2000 c. p. s. the number of steps that can be observed corresponds to the number of sound waves (counting only the negative peaks) from the beginning of the tone-pip to the largest negative peak. At 8000

RESPONSES TO TONE·PIPS BASAL TURN OF GUINEA PIG'S COCHLEA

FIGURE 4
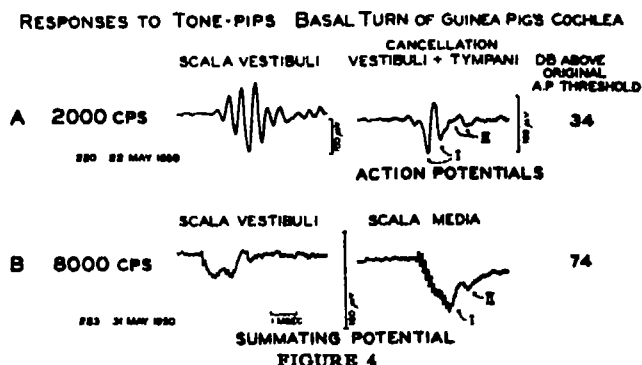
A. Cancellation of cochlear microphonic of 2000 c. p. s. tone-pip (gains adjusted to give minimum residue) reveals two volleys of action potentials (I) separated by one wave-length of the stimulating frequency. They are preceded by small waves of summating potential and cochlear microphonic in uncertain proportion. The late waves (II) include the second neural response (from the cochlear nucleus in the medulla).

B. Responses from scala vestibuli and from scala media (slightly retouched). No cancellation. In this experiment the cochlear microphonic disappeared almost completely when an electrode was inserted into scala media. Small action potentials are visible and the 8000 c. p. s. ripple is partly cochlear microphonic but the main deflections are summating potential. An upward deflection means cochlea more positive to reference electrode.

c. p. s., however, the shortening of latency as intensity increases seems to be continuous. More important, the total shortening as the stimulus is increased from threshold to a rather high level may be greater by at least two wave-lengths than the time from the beginning of the pip to the largest wave. It seems, therefore, that at threshold the process of stimulation must have been completed by a wave later than, and therefore smaller than, the maximum wave at the center of the tone-pip. We believe that this is good supporting evidence for the existence of summation, although

the argument makes the tacit but unproved assumptions that the conduction time for the nerve impulse and also the time required for the excitatory process to set off a nerve impulse are the same whether the stimulus be weak or strong.

The Summating Potential.—About a year ago[6] we described what we called "rectification" of the cochlear microphonic. Under several adverse circumstances, such as anoxia, the electrocoagulation of part of the cochlea, or the insertion of several electrodes deeply into the cochlea, the cochlear microphonic may apparently shrink to a few per cent of its original amplitude. The remaining response is "rectified," meaning that only one-half of each wave (the half in which scala vestibuli becomes electrically negative) still remains. This description is adequate for frequencies below 2000 c. p. s. We have recently observed that at higher frequencies there is also a shift of the base line in the direction of negativity in the scala vestibuli. The negative deflection appears to be produced by fusion of successive electrical pulses that recur with the frequency of sound waves. With an 8000 c. p. s. tone-pip the pattern resembles the negative half of the envelope of the pip, with an 8000 c. p. s. ripple also clearly visible (see Fig. 4). These "rectified" potentials are actually quite independent of the cochlear microphonic. They represent a third electrical potential of the cochlea which shows the property of summation at frequencies above 2000 c. p. s. This third potential decays rapidly enough, however, so that there is usually no effective carry-over (summation) below 2000 c. p. s. The maximum voltage of the third potential has not yet been measured because of serious technical limitations but it is well over 50 microvolts (scala media vs. reference electrode).

Evidence that the summating potential differs from both the cochlear microphonic and the action potential is as follows:

1. The summating potential (SP) is unidirectional (like the action potential) for any particular pair of electrodes. The cochlear microphonic (CM), on the other hand, is a change of electrical potential both above and below the original resting level.

2. SP shows summation. It outlasts the mechanical movement (unlike CM) and it shows no all-or-none behavior or refractory period (unlike action potentials (AP)).

3. SP and CM are differently oriented anatomically. The most favorable combination of electrodes for recording SP is scala media vs reference electrode. The most favorable combination for CM is scala vestibuli vs. scala tympani. Figure 1 shows how these axes are approximately at right angles to one another.

4. . The anatomical location of SP is apparently in the organ of Corti (like CM) and not in the spiral ganglion (unlike AP). AP appears equally and in the same direction at electrodes in scala vestibuli, media or tympani

(vs. reference electrode). SP appears strongly in scala media (vs. reference) but may be completely absent in scala tympani (unlike CM and AP), or, if the electrode is placed as far as possible away from the basilar membrane, its sign may actually be reversed. The possibility of placing an electrode on the positive side of the isopotential line in scala tympani shows that the origin of the potential difference must be well out in the cochlear canal and not within the modiolus.

5. The SP lags in time (or phase) behind CM. If CM from scala tympani is used to cancel CM from scala vestibuli, some SP as well as AP therefore remains (see Fig. 4 *A*). The lag seems to be of the order of 100 microseconds.

6. The SP may remain in the face of adverse conditions, such as anoxia or operative trauma, after the cochlear microphonic has virtually or completely disappeared. Action potentials have been observed under such conditions of operative trauma. This point is of special importance as it shows that *neither the summating potential nor the action potentials are in any way dependent on the cochlear microphonic.* The cochlear microphonic may be a good indicator of the time and a fair indicator of the intensity of mechanical movement of the organ of Corti, but it is not part of the chain of events that generates the nerve impulse.

7. At high intensities of stimulation, although still within limits perfectly tolerable to the human ear, the SP continues to increase with the intensity of the 8000 c. p. s. tone-pips, although the increase (like that of CM) becomes non-linear. While SP is still increasing CM reaches its maximum and may even begin to shrink again. It is worth noting that the behavior of SP is more in line with the behavior of AP, which is still increasing at this intensity, not to mention the sense of loudness to the human ear which is also still increasing.

8. Electrical polarization between scala vestibuli and scala tympani increases or decreases SP according to its direction. When the SP is increased the AP increases also. When SP is diminished AP diminishes or vanishes. The same intensity of electrical polarization causes a small, but only very small, change in the cochlear microphonic. If the cochlear microphonic is already absent it may be restored (or an equivalent microphonic may be introduced) by electrical polarization. The polarity of the microphonic relative to the sound wave will in this case depend on the direction of the polarizing current. We have not been able to reverse SP by polarization.

9. On the death of the animal AP disappears first; SP continues for a time after death; CM may continue longer, although at the low postmortem level that is already so familiar.[7] Sometimes SP outlasts CM.

Interpretation of the Summating Potential.—We believe that the summating potential arises in the terminal twigs of the auditory nerve fibers

which are arranged like baskets around the hairless ends of the hair cells. We believe also that the summating potential is a local excitatory process analogous to the end-plate potential at the neuro-myal junction and to the post-synaptic potentials of spinal neurons.[8] How the mechanical movement sets up the local excitatory process (the summating potential) is unknown.

The latency of the action potential relative to the mechanical movement we interpret as due mostly to conduction time from the nerve endings to the cell bodies. The shortest latency that we have measured for the foot of the action potential from the beginning of the cochlear microphonic following a strong, sharp click (rarefaction) is 550 microseconds.

General Significance.—We have described a summating potential which seems to represent the excitatory process initiated by the sensory cells of the cochlea in their afferent nerve fibers. Similar electrical potentials, probably representing the same type of excitatory process, have been recorded from muscle spindles[9] and from Pacinian corpuscles,[10] and by analogy we may expect to find them also in the related sense organs for touch, pressure, vibration, muscle sense, orientation to gravity and acceleration. The summating potential in the ear and perhaps elsewhere should be a useful tool for determining the mode of action of drugs, fatigue, etc., on the sense organ and for investigation of the process whereby mechanical force initiates the excitatory process.

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SEXUAL DIFFERENTIATION IN RHABDOSTYLA VERNALIS*

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Communicated by T. M. Sonneborn, July 29, 1950

Introduction.—The problem of sex in the protozoa has claimed the attention of many investigators. The most fruitful explorations of the problem stem from the studies on Paramecium made by Sonneborn,[1] Jennings,[2] and their colleagues. Working in a similar subject matter area, but with a different protozoan, Finley[3] ascertained the origin of conjugating individuals of *Vorticella microstoma* and reported evidence regarding their sexual differentiation. In *V. microstoma* conjugation was found to be a sexual type of reproduction which involved the union of a micro- and macroconjugant, the two organisms becoming one entity and offspring being produced therefrom. A preconjugation fission was described and interpreted as the conjugant (sex ?) differentiating fission.

This investigation was undertaken to extend the knowledge of conjugation in peritrichous protozoa. It was believed that new findings would be of considerable importance in understanding sexual differentiation in protozoa and might lead to contributions properly bearing upon broad problems of sex.

Rhabdostyla vernalis was investigated by methods similar to those previously reported by Finley.[3] Results obtained thereby indicate that sexual differentiation in Rhabdostyla is comparable but not identical to the phenomenon discovered in Vorticella.

Experimental.—A parental stock of Rhabdostyla was established by allowing a single individual to multiply vegetatively. Its progeny were used throughout the course of this study. The organisms were cultivated in Columbia culture dishes from which other kinds of protozoa were meticulously excluded. The cultures were stored in covered moisture chambers. Subcultures from the parental stock were made by transferring approximately twenty-five telotrochs to a sterile dish containing sterile culture medium. This method of establishing subcultures made it unnecessary to inoculate the new medium with bacteria (which served as food for Rhabdostyla) since adequate numbers were carried along with the telotrochs.

Occasionally encysted specimens were obtained by the well-known method of sealing the culture vessel with a glass cover on which petrolatum had been smeared in order to exclude air. Encysted specimens were induced to excyst then allowed to multiply until their progeny entered into an epidemic of conjugation. However, the most reliable method for inducing conjugation was the following one: Old cultures containing sparse

animated populations and a few encysted Rhabdostyla (in a state of suspended animation) were activated by decanting the old culture medium and replacing it with three changes of distilled water; the water was replaced by a sterile culture medium consisting of equal parts of 1% aqueous solution of cerophyl,[4] filtered casein broth containing salts customarily included in Knopf's solution, and distilled water. Successful activation of the cultures resulted in a series of rapid vegetative fissions which continued for approximately 12 to 36 hours and culminated in the unique preconjugation fissions. Preconjugation fissions yielded macro- and microconjugants.

Live specimens were studied with the aid of widefield microscopes and also compound research type microscopes. The usual methyl-green-acetic and Feulgen and Heidenhain's preparations were studied. The abundant supply of material at hand made it possible to observe isolated specimens or mass cultures in the various stages of reproduction. Fortunately, this was done almost at the will of the observers.

Observations.—In the main, nuclear and cytoplasmic phenomena of *R. vernalis* were similar to those reported for *V. microstoma*. During vegetative and preconjugation fissions the macronucleus underwent a non-mitotic division. The micronucleus divided by mitosis. Each daughter product of vegetative fission received, approximately, an equal volume of the macronucleus and the cytosome. Each microconjugant got only $1/_3$ as much macronuclear and cytosomal material as its sister macroconjugant. The micronuclear material seemed to be equally distributed during the progress of preconjugation and vegetative fissions.

There was no apparent morphological difference between rhabdostylas about to undergo vegetative fission and those on the verge of preconjugation fission. Morphological differences between macro- and microconjugants appeared only when the preconjugation fission was well underway.

Each "neuter Rhabdostyla" usually produced one macroconjugant and four microconjugants, in contrast with one of each from *V. microstoma*. That difference can be explained. Rhabdostyla's microconjugant remained attached to its sister macroconjugant at the conclusion of unequal (preconjugation) fission and after a lapse of 5 to 10 minutes it began a fission which yielded two microconjugants of equal size. These two specimens, while still attached to the macroconjugant, divided by equal fission, the result being four microconjugants attached near the basal region of the macroconjugant. All four microconjugants developed a posterior ciliary wreath, detached themselves one after another and swam away.

The fate of microconjugants was determined to be either conjugation or death. Macroconjugants had a different fate. They were attractive to

microconjugants about two hours; if they were not conjugated within that period they lost their attractiveness and vegetative fission ensued.

We were unsuccessful in our attempts to induce macroconjugants to form protective cysts. Neither have we discovered evidence of autogamy. This strongly suggests that under ordinary circumstances their fate is either conjugation or vegetative fission. In either case, they possess a great progeny potential. When a successful union was established between macro- and microconjugant the usual vorticellid type of conjugation occurred. As previously indicated, an epidemic of conjugation may be imposed upon the progeny of vegetative forms.

The organisms produced by preconjugation fission were the only ones which permanently fused and conjugated. It was relatively easy to prove this fact by isolation experiments. When microconjugants were given ample opportunities to attach themselves to vegetative individuals they failed to do so, even though death was their only apparent alternative.

Summary.—Rhabdostyla's micro- and macroconjugant are derived from a "neuter individual" through an equal division of the micronucleus accompanied by an unequal division of the macronucleus and cytosome. The two products of this fission have different immediate fates although both are potentially destined for conjugation. The larger product is capable of participating in fruitful conjugation without further differentiation. In this respect *R. vernalis* and *V. microstoma* are alike. The smaller product of preconjugation fission is irreversibly destined to produce microconjugants, this being accomplished by two fissions which occur consecutively. Rhabdostyla's microconjugant, unlike the microconjugant of *V. microstoma*, does not seem to be completely differentiated at the same time as its sister macroconjugant.

The situation wherein one undifferentiated microconjugant produces four functional microconjugants is strikingly reminiscent although obviously not identical to the case where a metazoan's primary spermatocyte divides to form two secondary spermatocytes, and they in turn divide to form four spermatids which become functional spermatozoa.

This preliminary résumé of our results will be followed by a more detailed account to be published elsewhere.

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[4] Cerophyl is manufactured by Cerophyl Laboratories, Inc., Kansas City, Mo.

ON THE CYTOPLASMIC NATURE OF "LONG-TERM ADAPTATION" IN YEAST*

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Introduction.—The ability of substrate to influence the segregation of the capacity to form the corresponding adaptive enzyme system was indicated[1,2] by experiments designed to investigate this possibility in the case of melibiose fermentation in yeast. In this study a heterozygote was employed which regularly yielded the expected 2:2 ratio of positives to negatives in the absence of substrate. However, when the heterozygote diploid was adapted to melibiose and the cells kept in contact with the substrate during all subsequent procedures, the 4 haplophase segregants yielded a 4:0 ratio of fermenters to non-fermenters in 6 out of 7 instances.

A fundamental assumption of these studies was that the diploid heterozygote involved the mating between positive and negative spores differing in a single gene. It was further assumed that the negatives carried a recessive allele incapable of initiating the formation of the adaptive melibiose system. On the basis of these assumptions and the results obtained in the presence of substrate, it was concluded that, once initiated by the corresponding gene, enzyme formation could continue indefinitely in its absence. Lindegren[3] drew similar, though not identical, conclusions from an analysis of the segregants from a diploid heterozygotic for galactose fermentation.

Winge and Roberts[4] published results on what they call "long-term adaptation" which confirmed the apparent ability of substrate to modify the Mendelian ratios expected in the segregation of a diploid heterozygotic for a single gene. These authors, however, proposed a different explanation and one which they implicitly assume avoids the necessity of invoking transmissible cytoplasmic determinants of enzyme formation.

These authors suggest that their own results, as well as the previously cited melibiose experiments, are consistent with the concept that crosses

between negatives and positives with respect to carbohydrate fermentation in the yeasts do not always involve absolute genetic negatives and positives in the sense of complete absence of functional activity for the former, and presence of full functional activity for the latter. They would assume rather that wherever substrate can modify observed segregation ratios one is dealing with an instance in which a mating is being made between "slow" (the negative strain) and "fast" (the positive strain) genes. On this basis, after sufficient time has elapsed in contact with substrate, it would be impossible to distinguish the two strains since both would exhibit full enzyme activity. The ratios observed would therefore depend on the previous history of contact with substrate.

There is, however, one feature about the phenomenon of long-term adaptation as reported by Winge and Roberts, and confirmed during the course of the present investigation, which is not easily explained by the "slow" gene hypothesis. Once a slow adapting stock has become phenotypically positive in terms of enzyme activity it remains so indefinitely on continued subculture in the presence of the adaptive substrate and can maintain the character for a varying number of cell generations even in the absence of substrate.

One must conclude consequently that two changes have occurred during the 7-day adapting period required to convert the slow strain to a positive. One is, of course, the accumulation of sufficient enzyme to exhibit enzyme activity. The other is that the rate of enzyme formation has been greatly increased and that this augmented capacity for enzyme formation is inherited during subsequent vegetative generations.

In any phenomenon involving the appearance of a heritable modification it would seem necessary to rigorously rule out mutation and selection as the operating mechanism before entertaining other hypotheses. This is particularly essential in connection with long-term adaptation since it is always accompanied by considerable growth of the negative phenotype during the preadaptive period.

The experiments offered by Winge and Roberts on this issue, while highly suggestive, are not adequate for a decisive conclusion. Reversible loss and gain of a populational character under specified alterations of environmental conditions do not preclude the involvement of mutation and selection.[5] Further, the fact that 12 cultures derived from 12 distinct single-cell isolates behaved similarly is likewise not conclusive. By the very nature of the test for phenotype, scoring in fermentation tubes cannot be made until a population density of between 1×10^7 and 1×10^8/cc. is achieved. By this time, under the selective conditions which exist, and with a mutation rate of reasonable frequency, the populations might well be similar in composition quite independently of the nature of the single cell from which they were derived.

It is clearly essential to analyze what occurs within the population during the 6–7 days prior to the appearance of activity. The first question to be answered is whether or not any heterogeneity with respect to enzyme producing capacity exists within the population for unless this is present, mutation and selection cannot be considered as a descriptive mechanism of the phenomenon. If heterogeneity can be established it then becomes pertinent to inquire into its origin.

The present paper presents some of the results of an investigation of the basic mechanism underlying "long-term" adaptation to galactose. We shall confine ourselves here only to those results directly relevant to the origin and transmission of the capacity to form enzyme rapidly.

The results indicate that it is not a mutational phenomenon since a variance analysis performed according to the method of Luria and Delbrück[6] indicates that the heritable modification does not occur in the absence of substrate. Further, reversion from the fast to the slow phenotype on growth in the absence of substrate can be shown to be a mass change, occurring in over 90 out of a hundred cells at the time of reversion.

While the present investigation was in progress, Mundkur and Lindegren[7] published a paper in which they concluded that the phenomenon of long-term adaptation was mutational. They report the data of a variance analysis but do not calculate the variance. Calculation from their data yields a variance of 27.1 with a mean number of "mutants" per sample of 9.6. Assuming their estimated "mutation" frequency of 1×10^{-7} they should have obtained a variance in the neighborhood of 4000, if a mutational process were operating. Thus, if any conclusion is derivable from this experiment it is that "mutation," independent of the presence of galactose, is not involved.

Unfortunately, no conclusion can with certainty be drawn from their published experiment since none of the conditions necessary for the performance of a variance analysis of this kind were satisfied. The absence of a large variance under these conditions signifies nothing.

Methods.—(A) *Strain:* The yeast strain C_1d employed in these studies was a representative of the same species used by Winge and Roberts, Saccharomyces Chevalieri. It was derived as a single clone isolate from culture number NRRL-Y1345 supplied through the courtesy of Dr. L. J. Wickerham of the Northern Regional Laboratory. Under comparable circumstances, the behavior of this strain with respect to galactose fermentation was the same as that reported by Winge and Roberts.

(B) *Media:* The basic medium was made by adding the following to 1 liter of H_2O; Bacto-peptone, 5 g.; $MgSO_4$, 1 g.; KH_2PO_4, 2 g.; Difco-yeast extract 2.5 g. Agar (20 g.) was added when a solid medium was required. This medium, containing no added carbohydrate, is referred to in the text as B-medium.

Glucose medium contained, in addition to the above, 40 g. of glucose. The galactose medium was made by adding 20 g. of purified galactose to a liter of B-medium. Merck's galactose was purified by recrystallization from 70% alcohol. The use of purified galactose was found necessary for adequate control over the quantitative results obtained since the small amount of glucose normally present as a contaminant in galactose preparations modified the results considerably.

Eosine-Methylene Blue (EMB) test plates were made by adding 5 cc. of 0.5% Methylene Blue and 20 cc. of 2% Eosine to a liter of the galactose agar medium.

(C) *Adaptation Times:* Tests for adaptation times were carried out with Durham tubes, a culture being scored as positive when active release of gas into the inverted vial was noted. With this method a "fast" culture appears as positive in 1–2 days, whereas, a "slow" culture takes 5–7 days. Enzyme activity was assayed manometrically with the Warburg apparatus. The rate of anaerobic CO_2 release from galactose was taken as a measure of the activity level of the adaptive enzyme.

(D) *Counts:* Direct counts were made with an hemocytometer. Viable and differential counts were made by the use of spread plates. Aliquots ranging from 0.05 to 0.2 cc. were uniformly spread on the surface of the agar with a sterile bent glass rod.

Experimental Results.—(A) *Phenotypic Composition of "Slow" Adapting Population:* Analysis of glucose grown cultures of the slow-adapting stock C_1d on EMB-galactose plates quickly revealed the existence of heterogeneity. Two types of clones were apparent after four days of incubation at 30°. The predominant type was small (about 1 mm. in diameter) and possessed the pink color characteristic of a non-fermenter. The other was larger (between 3 and 4 mm. in diameter) and possessed the heavy coloration characteristic of a fermenter. The behavior of these two types in Durham fermentation tubes paralleled their appearance on the test plate. The large pigmented form produced gas in 1–2 days, whereas the small pink clones took from 4–5 days. In subsequent discussions we shall refer to the large pigmented clones as "positive" and to the small pink as "negatives."

Since one is observing *clonal* characteristics on such a test plate, it follows that the differences between the two types must be heritable. This immediately raises the question as to the mechanism underlying the production of these two types.

Before undertaking the description of the experiments pertinent to this question, it is desirable to state briefly certain characteristics of these two clonal varieties. It was generally found that 24-hour glucose grown cultures of C_1d produced one positive clone for about every thousand cells plated. Analysis of the composition of positive clones by restreaking on

EMB-galactose test plates indicated that they were for the most part composed of cells capable of giving rise to positive clones. A similar examination of negative clones revealed that anywhere from 10–60% of their component cells could potentially give rise to positive clones. The presence of negative cells evidently inhibits the expression of the positive phenotype by potentially positive cells. This inhibitory action explains the sharp drop in percentage of positive colonies often observed in very heavily seeded plates, as is, for example, illustrated in the experiments of Mundkur and Lindegren[7] who inoculated as many as 2 × 10⁸ cells in a single plate. The inhibitory effect of negative cells also plays a role in determining the duration of the long-term adaptation process in liquid media.
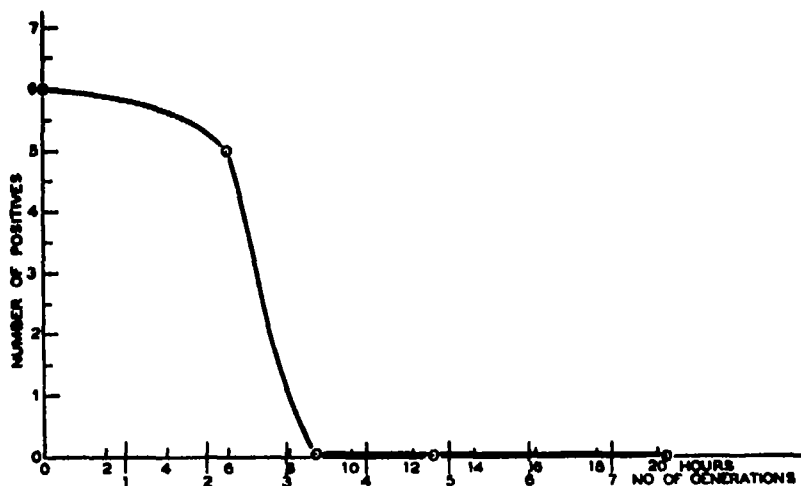
FIGURE 1

The effect of adding galactose at different periods of clonal development on the number of positive clones observed. Each point represents the average of three test plates to which galactose was added at the period indicated.

The ability of the potential positives to ultimately escape from this inhibition is demonstrated by the invariable heavy positive papillation which attends prolonged incubation of the negative clones.

(B) *Inhibition of Positive Phenotype by Negative Cells:* It was necessary to determine how early in the development of a clone the presence of phenotypic negative cells exerted their capacity to inhibit the expression of the positive phenotype. To do this, advantage was taken of the fact that an adaptive character was being followed, i.e., one that is not present in a cell prior to exposure to substrate.

The growth rate of 24-hour C_1d glucose-grown cells on solid EMB-B medium was determined by spreading a known amount of cells on the surfaces of a series of plates, which were then incubated at 30°C. for various periods of time. Subsequent to these periods of incubation, the cells were respread with 0.1 cc. of sterile water. The increased number of clones observed provided an estimate of the growth rate. Three separate determinations gave an average value of 0.38 generation per hour. Knowing the growth rate under these conditions made it possible to introduce substrate after a known number of divisions had occurred.

A series of EMB-B plates were seeded with 7000 C_1d glucose-grown cells. These were then incubated at 30°C. for various periods of time, subsequent to which galactose was added in the form of 4 cc. of a 10% solution in 2% agar. The plates were then incubated at 30°C. for 4 days and the number of positive clones counted.

The results of such an experiment are summarized in figure 1. The data indicate that if the clone is allowed to develop in the absence of the adaptive substrate for more than 3 generations the number of positive clones subsequently observed becomes negligible. These results were checked by another method which involved controlling the number of divisions of negative cells by the use of various concentrations of cyanide. These experiments gave essentially the same results, namely, that divisions which occur beyond the third generation do not detectably contribute cells which are capable of giving rise to phenotypically positive progeny in the clone within the four-day incubation period.

One important practical aspect of this result is that it permits the use of these test plates for a variance analysis since the divisions beyond the 3rd do not contribute significantly to the number of positive clones.

(C) *Variance Analysis of the Origin of Positives:* The method devised by Luria and Delbrück[6] to decide whether a particular "mutant" type arises randomly or is induced by the testing or selective agent depends on the distributions of mutant types in a series of separate cultures developed in the absence of the agent. The induction hypothesis would predict a distribution yielding a variance characteristic of the usual errors of sampling, i.e., the variance would be of the same order of magnitude as the mean. The mutational hypothesis, on the other hand, presumes that the heritable modification can occur in the absence of the agent and hence at any time in the history of the development of the separate cultures from which the samples are being taken. Accordingly, in sampling from such tubes, one counts not only the mutants but also all the progeny derived from them between the moment of mutation and the time the sample is removed. Under these circumstances, it would be expected that the variance of the distribution of mutants would be much larger than that due to sampling error. Luria and Delbrück[6] derive an expression which

permits one to estimate what they call the "likely" variance on the basis of the random mutation hypothesis.

Several criteria must be satisfied if a variance analysis of this nature is to be meaningful. The medium employed in the development of the separate cultures from which samples are removed must be one that permits equal growth rates of the normal and "mutant" types. The populations must not be allowed to go into the stationary phase where selective forces would be difficult to control and which might result in the elimination or severe suppression of one of the types being followed. The testing conditions which distinguish between the two phenotypes must be such that contributions of the mutant type due to growth of the negative under the testing conditions must not be significant. Finally, the

TABLE 1

VARIANCE ANALYSIS OF THE APPEARANCE OF POSITIVES IN SAMPLES OF SEPARATE CULTURES GROWN IN THE ABSENCE OF GALACTOSE

Exp. number	41	67	67a	95
Growth medium	Glucose	B	B	B
Test plate	EMB-PG	EMB-PG	EMB-PG	EMB-PG + NaCN
Number of cultures	10	10	20	10
Volume of cultures, cc.	1	0.5	0.5	1
Number of organisms/cc.	1×10^6	1×10^6	1×10^6	5×10^6
Volume of samples, cc.	0.2	0.1	0.1	0.4
Mean number of positives/sample	5.0	24.6	21.6	18.9
Observed variance	9.1	35.0	24.8	112.3
Estimated "likely" variance	2000	2000	4000	10,000

Variance of method	Multiple samples from same culture		
Volume of sample	0.2	0.1	0.4
Number of samples	5	10	10
Mean number of positives/sample	2.4	20.4	9.8
Variance	6.2	28.8	43.4

NOTE: All cultures incubated at 30°C. in standing tubes. See text for further details.

inoculum used in seeding the separate tubes must be low enough so as to insure against the probability of seeding a mutant cell initially and, at all costs, the seeding of an equilibrium population of mutant and normal types must be avoided.

It was found that the B medium, as well as the glucose medium, satisfied the first criterion mentioned. The former supported a growth rate equivalent to 0.34 generation per hour and the latter 0.67 generation per hour for both types. In the series of experiments to be described, the populations were sampled when they reached the level of approximately 1×10^6 organisms per cc., which is well below the stationary populations achieved in either of the two sorts of media employed. In view of the results in the

previous sections, it is evident that the EMB galactose plate can be used as a test plate for such analysis. Finally, in all the experiments recorded, the separate cultures were seeded with between 1 and 10 cells each, so that the probability of seeding a mutant was small and the chances of seeding an equilibrium population were nil.

An estimate of the apparent "mutation" frequency was made by the "median" method of Lea and Coulson[6] and yielded an average value of 5×10^{-4} "mutations" per cell per division. Using this figure, it was calculated that the employment of 10 separate cultures, allowed to grow up to a population density of about 1×10^6 per cc. should yield a "likely" variance of 2000 and over if mutations leading to greater capacity for enzyme formation were occurring in the separate cultures without substrate.

Table 1 summarizes a series of variance determinations performed under the conditions noted above. The first three columns present three such determinations which employed the EMB galactose test plates. The last column, Exp. 95, is one of a series of experiments testing the adequacy of the EMB galactose plate by employing a test plate which prevents significant growth of the negative type. A negative cell in contact with the pure galactose medium must depend primarily upon oxidative metabolism for growth. Consequently, the introduction of anaerobiosis of various degrees limits the capacity of such cells to develop. Cells which adapt, however, can continue to grow and give rise to macroscopic clones under such circumstances. The usual anaerobic jars were not found suitable and recourse was had to the use of sodium cyanide. The cyanide was added with the cell suspensions as they were spread and the plates were then sealed individually with either plasticine or Scotch tape. This sealing was essential in order to retain the cyanide during the incubation. It was empirically established that 0.5 cc. of 10^{-1} M sodium cyanide added in this manner was sufficient to prevent significant growth of the negatives and yet permit the adaptation and growth of the positives.

It is evident from the data that the observed variance in the number of positives is not very much different from the mean and certainly very much below that expected from the mutational hypothesis. The variance of the method, determined from a number of samples from the same tube, is included in the lower portion of table 1. These are of the same order of magnitude as the experimental variances observed from the different cultures. It will be noted that the variance of the cyanide method is higher than that obtained with the plain EMB galactose plates. This is a reflection of the difficulty of maintaining identical concentrations of cyanide in the separately sealed plates during the incubation period. Such variation from plate to plate increases the variance above that to be expected from the error of sampling. This higher variance is also of course seen in the experimental samples derived from the separate cultures.

The experimental variance is nevertheless proportionately as far below the "likely" variance as the others and furthermore agrees with the variance of the cyanide method itself.

It seems necessary to conclude from these data that "mutations" to the positive phenotype do not occur in cultures developed in the absence of
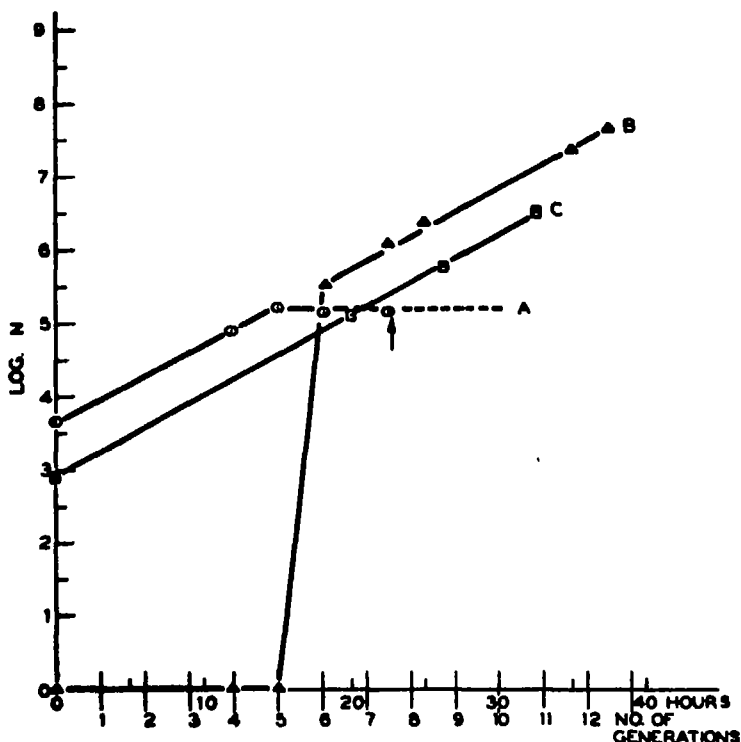
FIGURE 2

The reversion of positive cells to the negative phenotype during growth in the absence of substrate. Curve *A* represents the number of positives, and curve *B* the number of negatives during the reversion. Curve *C* represents the growth of negative cells alone in the same medium under the same conditions. The arrow indicates the point at which a transfer was made to a fresh tube in the reversion experiment.

substrate. Thus, the heritable modification leading to rapid enzyme production which occurs in certain cells during long-term adaptation is induced by the adaptive substrate.

(*D*) *Reversions of Positives to Negatives:* The next question requiring analysis was the mechanism underlying the reversion of "fast" cultures to the original "slow" type, during growth in the absence of substrate. Here

again, use was made of the EMB-galactose test plate to examine the details of the conversion.

The irregularities in the times required for reversion as recorded by Winge and Roberts[4] suggested the desirability of studying this phenomenon under relatively well-controlled conditions. In particular, it seemed preferable to begin the examination with positive clones which were comparable in their adaptive history. To accomplish this, positive clones were chosen which had arisen on EMB-galactose plates as a result of spreading a glucose-grown C_1d culture never previously exposed to galactose. Such clones were suspended to a density of 1×10^4 organisms per cc. in 5 cc. of B-medium contained in test tubes. These were incubated at 30° C. and samples removed at intervals for plating on EMB-galactose plates. To avoid the complications which ensue in stationary phase populations, transfers were made when the density reached about 5×10^6 per cc. so as to restore the population to between 1×10^4 and 1×10^5 organisms per cc.

The results obtained in such experiments were extremely uniform and are typified by the experiment described in figure 2. Curve C represents the growth curve of the negative type in the same medium under identical circumstances. Curve A represents the behavior of positives during the course of the experiment. It will be seen that they grow perfectly normally up until about the fifth generation, and then suddenly cease to increase. Curve B, which describes the appearance of negatives, shows that the number of negatives is quite negligible prior to the fifth generation. Then quite suddenly, they appear in numbers equal in magnitude to the number of positives present in the culture at the time. The burst in the appearance of negatives, is, in these experiments, always coincident with the cessation of the increase in the number of positives. Subsequently the negatives grow at their characteristic rate.

It would appear that the positive cells are capable of producing positive buds for about five generations. Quite suddenly, however, the vast majority of the positive cells begin to produce negative buds. Despite the fact that they are producing negative buds it is apparent from the constancy in number of positives that they are still phenotypically positive in the sense that they can give rise to positive clones when brought into contact with substrate on a test plate. It was not possible in this particular experiment to determine the ultimate fate of these positives. Other experiments indicate that they do not remain as such indefinitely. It has proved experimentally difficult to decide, however, whether their final disappearance is due to death or conversion to negatives.

The possibility that the burst of negatives observed in this and other similar experiments may be due to a marked, but transient, stimulatory effect of the presence of positives on the growth of negatives can be ruled

out on several grounds. In the first place, the apparent stimulation leads to growth rates too high to be reasonably attained, being, in some experiments, 20 times the normal growth rate of the negative type. Further, reconstruction experiments in which the growth of negatives was followed in artificial mixtures of positives in varying proportions exhibited no such stimulation prior to the 5th generation, the normal time of reversion of the positives. Finally, the number of negatives which appear in any given experiment at the burst is quantitatively related to the number and the growth rate of the positives present at that particular moment. This is illustrated by the data presented in table 2 which presents results of representative reversion experiments. In this table are calculated the growth rates in terms of generations per hour of the positives in the period prior to the burst and of the negatives in the period beyond the burst. The "apparent" growth rate of the negatives during the burst is calculated on the assumption that negatives derive only from negatives. It will be seen that these "apparent" growth rates range anywhere from 6 to 16 times the normal growth rates of the negatives. A "corrected" growth rate was

TABLE 2

COMPARISON OF GROWTH RATES (GENERATIONS/HOUR) OF POSITIVES AND NEGATIVES DURING VARIOUS PERIODS OF A REVERSION EXPERIMENT

EXP. NO.	POSITIVE IN PRE-BURST PERIOD	NEGATIVE IN POST-BURST PERIOD	POSITIVE AT BURST	—NEGATIVE AT BURST—	
				APPARENT	CORRECTED
118	0.37	0.36	0.01	4 73	0 39
121a	0.36	0.36	<0	2 13	0.35
121b*	0.17	0.19	0.001	1 41	0.16
127	0.38	0.37	0 003	5.90	0 36

* NOTE: "Apparent" growth rate at burst was calculated with the assumption that negatives are derived from negatives. "Corrected" growth rate determined on the assumption that negatives are derived from negatives and positives. All experiments except 121b were performed at 30°C. The latter was carried out at 14°C.

calculated on the assumption that the negatives during the burst period are derived both from the small number of negatives present as well as from the positives. It is seen that the growth rates thus obtained are, within experimental error, identical to the growth rates of the positives and the negatives during periods when each is derived from its own type.

The good agreement among the various growth rates indicates that the production of negative progeny by the positive cells is proceeding at the normal division rate of these cells and that the vast majority of the positive cells present participate in the production of negative buds.

Experiment 121b, which was performed at 17°C. rather than 30°C., offers further convincing evidence for these conclusions. At this temperature, the growth rates of the positives and negatives are less and the corrected growth rate of the negatives during this period corresponds to the growth

of the positives at this lower temperature. It is noteworthy that the number of generations required for the burst is not significantly different at the slower growth rate, although the time of its occurrence is of course about doubled.

So long as attention is confined to recently derived positives, the results obtained in reversion experiments are quite uniform. The reversions are massive ones involving most of the cells present and generally occur between the 5th and 6th generations. The number of generations required for the reversions has been, within the range studied (0.20 to 0.67 generations/hr.), relatively independent of the division rate.

When, however, the reversion phenomenon is studied with clones derived from cultures subjected to prolonged growth in the presence of galactose, great variability is observed both in the number of generations required for the burst and in the percentage of individuals participating when it does occur. The irregularity exists not only among cultures undergoing independent serial passage in galactose but also among the individual cells of a given culture. Thus, one clone from a culture may not revert even after 70 generations whereas another may show evidence of reversion after 6 generations. , Little can as yet be said about the nature of this irregularity except that it is evidence for some discontinuous process affecting the ability to preserve the capacity of rapid enzyme formation during growth in the absence of substrate. In general it may be noted that serial subculture in the presence of substrate increases the number of individuals requiring a large number of generations before reversion occurs. It is this phenomenon which is undoubtedly the basis for the irregularity observed by Winge and Roberts[4] in their reversion experiments.

Discussion.—(A) Nature of the Phenomenon at the Populational Level: Winge and Roberts[4] explain the long delay preceding the appearance of enzyme activity by assuming that they are dealing with cells which form enzyme slowly at a rate determined by the possession of a slow gene. Aside from the fact that it fails to provide an explanation of the heritable nature of the adapted state it is evident that the above hypothesis is inconsistent with the data obtained in the present analysis.

Account must be taken of the demonstrated heterogeneity in the original population. Under the experimental conditions described, there are two factors which appear to determine the duration of the adaptation times in such slow strains. .

(1) Only a small proportion of the individuals seeded are actually capable of undergoing the adaptation.

(2) The presence of phenotypic negatives inhibits the complete expression of the positive phenotype.

A simple calculation makes it evident that the second factor is a quantitatively important one. From the viewpoint of the first factor, the

difference between seeding a thousand cells of a "slow" stock and a thousand cells of a "fast" stock is that, in the former, one is seeding only one adaptable cell and in the latter a thousand. With a generation time of about 0.67 generation per hour this thousandfold difference in initial inoculum should be made up in 10 generations or about 16 hours. It is clear that this alone does not explain the 4–5 day difference in the adaptation times between fast and slow stocks. Consequently the inhibitory mechanism forms a quantitatively important component of the phenomenon of long-term adaptation.

It should further be noted that the actual speed of adaptation of those cells in a "slow" population which do adapt compares favorably with the rate of adaptation observed with cells from fast stocks. The experiments cited indicate that adaptation occurs within 3 generations, i.e., 10 hours or less. This compares with 5 hours required for full adaptation in cells from fast stocks.

(B) *The Nature of the Phenomenon at the Cellular Level:* The evidence presented indicates the involvement of a heritable modification in rate of enzyme production induced by the presence of substrate. The mass reversion which occurs on growth in the absence of substrate makes it highly unlikely that a substrate-directed genic change is involved, and in any case, removes the criterion of genic stability necessary for testing this possibility by classical genetic methods.

Under these circumstances it seems difficult to avoid looking for a cytoplasmic basis to explain the heritable nature of the change. All the data thus far obtained on the phenomenon can be adequately explained by the existence of cytoplasmic units possessing the following properties: (1) they are concerned with enzyme formation; (2) their number increases more readily in the presence of substrate; (3) their law of growth is autocatalytic.

Previous studies[9]–[11] of enzymatic adaptation in resting cells of "fast" strains have revealed that the kinetics of enzyme formation are autocatalytic in nature and that the enzyme forming systems interact in a competitive manner. These and other results were summarized[9, 10] in a concept of gene control over enzyme synthesis which assumed that (a) cytoplasmic units (plasmagenes) exist which mediate the formation of specific enzymes, (b) substrate stabilizes the enzyme-forming complex and thus leads to an increased net rate of formation of both enzyme and cytoplasmic unit; and finally (c) the law of growth of these elements is autocatalytic.

It is evident that the conclusions derivable from the investigations of both normal and "long-term" adaptation are similar. The latter phenomenon, involving as it does transmission during cell generation, represents more decisive evidence for the autocatalytic growth of the cytoplasmic

determinants.   In addition, the discreteness exhibited in both the appearance and loss of the ability to transmit enzyme forming capacity revealed the particulate nature of the underlying mechanism with a clarity not possible with the fast adapting strains.

In this connection it should be noted that the dramatic suddenness with which the production of negative progeny ensues finds ready interpretation in the mode of cell production characteristic of the yeasts.   The fact that a yeast bud receives only 10% of the parent cytoplasm has two important numerical consequences for cytoplasmic transmission.   One is that the probability of the daughter cell obtaining a particular cytoplasmic element is 1:10 rather than 1:2 for the usual mechanism of cell division.   The second is that each cell generation results in a 1:10 dilution of parental cytoplasm.   Thus, by the 5th generation in a yeast clone, a 1:100,000 dilution has occurred as compared with a 1:32 dilution in a form such as paramecium.

The crux of the problem has always been, and still is, the nature of the particulate elements involved and the degree of autonomy to be ascribed to them by virtue of their behavior and autocatalytic growth.

Perhaps the simplest explanation of the autocatalytic behavior is one which depends upon the fact that the enzyme being followed is one which is involved in the energy generating mechanism.   Thus the more enzyme present, the greater the possible rate of further enzyme synthesis.[10, 11] This suggestion has been made untenable by experiments with C_1d positives which will be detailed in a separate publication.   It was possible to demonstrate that cells in which none of the adaptive galactozymase could be detected still retained the capacity to form enzyme rapidly and furthermore could transmit this ability to their progeny.   It appears that particles other than the active enzyme molecules themselves are responsible for the ability to form more replicas.

The existence of a genic background to the phenomenon in terms of a single gene difference between the "fast" and "slow" varieties has been well established[4] and constitutes the only important difference from the studies of Sonneborn[12] and his collaborators on the inheritance of antigenic type in paramecia.   The reversion experiments exhibiting the production of progeny incapable of forming an enzyme which the parent cell can synthesize are very similar to the phenomenon reported by Ephrussi[13] and his coworkers on the inheritance of the ability to form cytochrome-oxidase and succinic dehydrogenase in yeast.   It should however be noted that in the latter instance the loss is permanent.   In the case of the adaptation to galactose it is not.   A certain small proportion of the progeny derived from a negative cell regain the capacity on being brought into contact with substrate and hence a replenishing mechanism must be presumed to exist.

Whatever the detailed mechanisms turn out to be, it seems likely that the phenomenon of long-term adaptation can provide an analytical tool which could help answer some of the questions raised by the previous studies on enzymatic adaptation. In principle it could provide information not easily attainable in any other way, for with its aid a particle analysis analogous to the admirable studies of Preer[14] and Sonneborn[15] in paramecia may now be feasible in the case of enzymatic adaptation in the yeasts.

Summary.—The phenomenon of "long-term adaptation" to galactose fermentation by yeast has been analyzed. The possibility that it is based on mutation and selection has been eliminated by a variance analysis and the demonstration that reversion to the original type is a mass phenomenon.

The data indicate that contact with substrate induces, in a small proportion of cells, a modification leading to ability to form enzyme. This modification is transmitted from one cell generation to the next by cytoplasmic elements which increase autocatalytically. These conclusions concerning the cytoplasmic factors determining enzyme formation are in agreement with those deduced from previous studies of enzymatic adaptation in fast adapting strains.

The importance of the phenomenon and its experimental analysis stems from the ability to thus exhibit the discrete nature of the cytoplasmic components determining enzyme synthesis. A more adequate quantitative approach to the nature of these units is thus made possible.

¹ Spiegelman, S., Lindegren, C C., and Lindegren, G , PROC. NATL. ACAD. SCI., 31, 95–102 (1945).

² Lindegren (*Cold Spring Harbor Symp. Quant. Biol.*, 11, 115–129 (1946)) reported difficulty in repeating the melibiose experiments. Subsequent publication in 1949 (*The Yeast Cell*, Educational Publishers, St. Louis, 1949, Chapter 26, p. 19) of the data on which these doubts were raised makes it possible to examine their relevancy to the earlier experiments. Several features make interpretation of these later experiments difficult and comparison with the previous results impossible. The cross employed in the original experiments consistently gave the normal 2:2 segregations of +: − in control matings. The "control" cross used by Lindegren for the repetition yielded only 1 normal 2:2 out of 9 complete tetrads analyzed. Five of the asci yielded a 1:3 ratio of positive to negatives and the remaining 2 asci did not possess the positive allele in any of the 4 spores! Furthermore, the plus and minus strains used in the experimental matings in the presence of substrate were not the same as those used in the experimental cross. It may well be questioned if these two sets of matings are at all comparable. Finally, if they are considered to be comparable, it seems necessary to entertain the probability that the presence of substrate had an effect on the segregation of positives. As compared with 1 out of 9 in the controls, all four complete tetrads analyzed in the experimental series yielded a 2:2 ratio. The probability of this being due to chance is in the neighborhood of 1 in 10⁴.

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NON-ENZYMATIC OXIDATION OF TYROSINE AND DOPA

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Introduction.—In the course of studies demonstrating the existence of tyrosinase activity in normal pigmented mouse skin, to be reported elsewhere, it was noted that under certain conditions not only dopa (3,4-dihydroxyphenylalanine) but also tyrosine was capable of undergoing non-enzymatic oxidation. The necessary conditions for the non-enzymatic oxidation of tyrosine involved the presence of both cupric ions and a "threshold concentration" of dopa. It was also observed that the oxidation of tyrosine ended even though most of the tyrosine remained in the system unaffected.

FIGURE 1

Simplified scheme for the non-enzymatic oxidation of tyrosine and dopa in the sequence leading to the formation of melanin.

The mechanism proposed to account for the non-enzymatic oxidation of tyrosine involves the role of dopa quinone as an electron and proton acceptor, of tyrosine as an electron donor and of cupric ion as an electron transporter. The over-all chemical reaction can then be written

$$\text{Tyrosine} + \text{Dopa quinone} + H_2O = 2 \text{ Dopa.}$$

The reaction sequence is then readily visualized as in the simplified scheme

shown in figure 1. On the basis of this scheme it is possible to perform a kinetic analysis with consequences that can be tested experimentally.

Kinetic Analysis.—From the system shown in figure 1 it is possible to set up the following rate equations (assuming that the reactions are first order with respect to the various reactants):

$$\frac{dQ}{dt} = -k_2Q - k_3QT + k_1D, \tag{1}$$

$$\frac{dD}{dt} = k_3QT - k_1D, \tag{2}$$

$$\frac{dT}{dt} = -k_3QT. \tag{3}$$

Equation (3) is readily solved for T to give

$$T = c_1e^{-k_3\int Q\,dt}. \tag{3a}$$

Substitution for T from equation (3a) in equation (2) gives

$$\frac{dD}{dt} = k_3Qc_1e^{-k_3\int Q\,dt} - k_1D. \tag{2a}$$

Integration of this linear equation gives

$$D = Ce^{-k_1t} + k_3c_1e^{-k_1t}\int Qe^{-k_3\int Q\,dt}e^{k_1t}\,dt. \tag{4}$$

Substituting the value for k_3QT from equation (3) in equation (2), we have

$$\frac{dD}{dt} + k_1D = -\frac{dT}{dt}. \tag{2b}$$

On integrating this linear equation, we have

$$D = T_0e^{-k_1t} - T + k_1e^{-k_1t}\int Te^{k_1t}\,dt. \tag{5}$$

Now, substituting for T from equation (3a) and for D from equation (5) in equation (1), we have

$$\frac{dQ}{dt} = -k_2Q - k_3Qc_1e^{-k_3\int Q\,dt} + k_1[T_0e^{-k_1t} - c_1e^{-k_3\int Q\,dt} +$$
$$k_1e^{-k_1t}\int c_1e^{-k_3\int Q\,dt}e^{k_1t}\,dt]. \tag{6}$$

It is possible to solve for the variables Q, D and T as functions of t, although with great difficulty.

Since, in the non-enzymatic experiments, the oxidation of dopa and tyrosine ends with most of the original tyrosine still present, a simpler approximate solution of the equations for Q and D may be obtained by assuming that the concentration of tyrosine remains constant. Thus the original equations become

$$\frac{dQ}{dt} = -k_2 Q + k_1 D \tag{1'}$$

and

$$\frac{dD}{dt} = -k_1 D, \tag{2'}$$
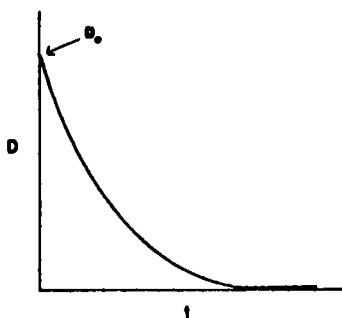
FIGURE 2

Graph of D as a function of t; equation (2'a).
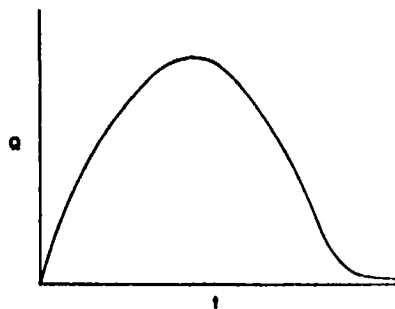
FIGURE 3

Graph of Q as a function of t, with coordinates for maximum value of Q given in equation (5'a) and equation (4'a). Here equation (3') is plotted.

since, from equation (3), $\dfrac{dT}{dt} = -k_2 QT = 0.$

Solving for D in equation (2') gives

$$D = D_0 e^{-k_1 t}. \tag{2'a}$$

This equation, when plotted, gives the familiar first-order curve shown in figure 2.

Substituting the value for D from equation (2'a) in equation (1'), we have

$$\frac{dQ}{dt} = -k_2 Q + k_1 D_0 e^{-k_1 t}. \tag{1'a}$$

On integrating this linear equation, and knowing that $Q = 0$ at $t = 0$, we have

$$Q = D_0 \frac{k_1}{k_1 - k_2} (e^{-k_1 t} - e^{-k_1 t}). \tag{3'}$$

The following deductions can be made from equation (3'):

1. Since Q cannot have negative values, $k_1 > k_2$.
2. $Q = 0$ at $t = 0$, and $\lim\limits_{t \to \infty} Q = 0$.
3. Since $Q = 0$ at zero and at infinite times, it must have a maximum value.

Differentiating equation (3'), setting the differential expression equal to zero and solving for t at the maximum for Q results in the following:

$$t_{max.} = \frac{\ln (k_1/k_2)}{k_1 - k_2}. \tag{4'}$$

If $k_1 \gg k_2$ (as will be justified later), then

$$t_{max.} = \frac{\ln (k_1/k_2)}{k_1}. \tag{4'a}$$

Now, substituting value for $t_{max.}$ from equation (4') in equation (3') and simplifying, we have

$$Q_{max.} = D_0 \frac{k_1}{k_1 - k_2} \left(\frac{k_1}{k_2}\right)^{k_1/(k_2 - k_1)} \left(\frac{k_1 - k_2}{k_2}\right). \tag{5'}$$

If $k_1 \gg k_2$, then

$$Q_{max.} = D_0. \tag{5'a}$$

Equation (3') is plotted in figure 3.

Further important conclusions, which can be checked experimentally, can result from an additional analysis if we make one assumption. This assumption is that the rate of melanin production is proportional to O_2 consumption as measured in a Warburg respirometer. This is justifiable since extraneous interfering or inhibitory substances are not present in the non-enzymatic system. Since

$$\frac{dM}{dt} = k_2 Q, \quad M = k_2 \int_0^t Q \, dt$$

Substituting value for Q from equation (3'), we have, on integration,

$$M = \frac{D_0 k_1 k_2}{k_1 - k_2} \left[\left(\frac{k_1 - k_2}{k_1 k_2} \right) - \left(\frac{1}{k_2} e^{-k_2 t} - \frac{1}{k_1} e^{-k_1 t} \right) \right]. \tag{6'}$$

Expanding the term e^{-kt} into a Maclaurin's series, and using the first three terms, equation (6') becomes

$$M = D_0 k_1 k_2 \frac{t^2}{2}. \tag{7'}$$

It should be noted that equation (7') is arrived at on the assumption that k_1 and k_2 are of the same order of magnitude.

If, however, $k_1 \gg k_2$, then equation (6') can be written, on simplification,
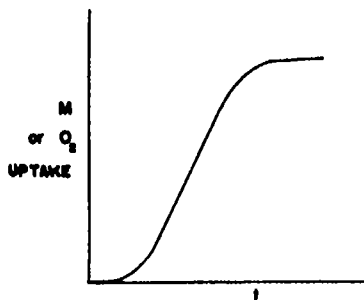
FIGURE 4

Graph of M or O_2 consumption as a function of t; equation (7').
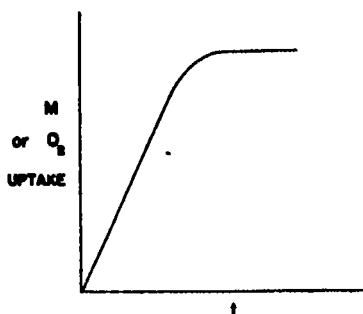
FIGURE 5

Graph of M or O_2 consumption as a function of t; equation (9').

$$M = D_0(1 - e^{-k_2 t}). \tag{8'}$$

Expanding the term $e^{-k_2 t}$ into a Maclaurin's series, and, using the first two terms, equation (8') becomes, finally,

$$M = D_0 k_2 t. \tag{9'}$$

Now, if equation (7') holds, i.e., if k_1 is not much greater than k_2, then, if M or O_2 consumption is plotted against t, the curve should show an induction period for small values of t, as in figure 4. If, however, equation (9') holds, i.e., if $k_1 \gg k_2$, then, if M or O_2 uptake is plotted against t, the curve should be a straight line for small values of t, as in figure 5. Now, actual measurements of O_2 uptake in Warburg respirometers result in the curve shown in figure 5, in the case of the non-enzymatic system,

Thus, the experimental evidence supports the conclusion that $k_1 \gg k_2$.

Discussion.—(A) Mechanisms other than the non-enzymatic oxidation of tyrosine to dopa proposed here have been considered and tested. For example, O_2 consumption in excess of the amount needed for complete oxidation of dopa can be attributed to the formation of hydrogen peroxide in the oxidation of dopa to the quinone stage. Furthermore, the oxidation of tyrosine might be an oxidative deamination, rather than an oxidation to dopa, with consequent increased O_2 consumption. The experimental evidence (to be reported elsewhere), however, is strongly against these other possibilities as explanations for *both* the excess O_2 consumption and correspondingly increased pigment formation observed.

(B) According to the scheme in figure 1, the reaction should not end until all the tyrosine is oxidized. The fact remains, however, that the reaction apparently ends despite the presence of a large amount of un-oxidized tyrosine. This is explained by assuming, that $k_1 \gg k_2 \gg k_3$, i.e., the rate of dopa formation is much less than the rate of dopa and quinone utilization in melanin formation. Thus the concentrations of dopa and of dopa quinone fall rapidly to a level at which the rate of dopa formation becomes immeasurably slow, and soon thereafter the reaction ends, for all practical purposes. Thus, the operational term "threshold concentration" actually means that concentration of catalyst sufficient to give a measurable reaction.

(C) Aside from the kinetic analysis previously presented, there are several other points of interest arising from a consideration of the non-enzymatic system in which both tyrosine and dopa undergo oxidation:

1. It is a system in which a side reaction has the features of an auto-catalytic reaction.

2. It provides (since tyrosinase is a copper enzyme) another example of cases reported by Smith and Lumry,[1] where reactions catalyzed by metal enzymes can be catalyzed by the specific metal ions alone, although in the instance reported here, the metal ion catalysis is far less effective and lacks the specificity of the enzymatic catalysis.

3. It may reflect some conditions in the more complicated enzyme system. Then tyrosinase may have a function in addition to its mono-phenolase and diphenolase activities; that is to say, tyrosinase may also act as an electron transporter between tyrosine and dopa quinone.

[1] Smith, E. L., and Lumry, R., *Cold Spring Harbor Symp. Quant. Biol., New York,* **14**, 168–178 (1949).

COULOMETRIC TITRATIONS WITH EXTERNALLY GENERATED REAGENTS

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Communicated by W. A. Noyes, Jr., September 12, 1950

The feasibility of several types of coulometric titrations in which the titrant is generated in the solution containing the sample has already been
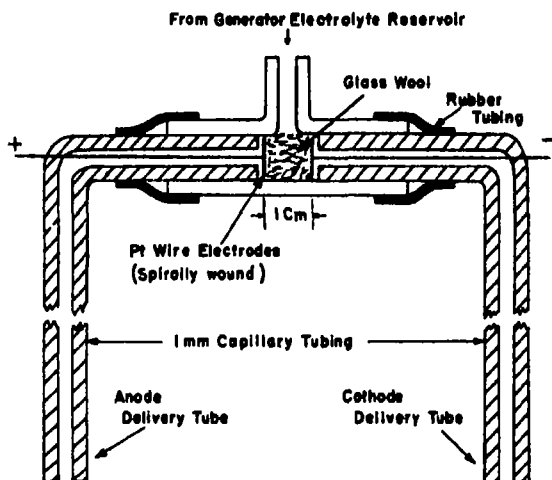
FIGURE 1

Generator Cell

demonstrated.[1,2] We have found that satisfactory coulometric titrations may also be performed by means of reagents which are electrolytically generated in a separate generator cell and subsequently delivered to the titration vessel.

SAMPLE	NO. OF DETNS.	MEQ. TAKEN	MEQ. FOUND	AVERAGE PRECISION, %	AVERAGE ERROR, %
HCl	4	1.208	1.211	0 1	+0 2
	3	2.417	2.429	0.1	+0.5
NaOH	5	0.841	0.843	0.2	+0.2
	3	1.682	1.689	0.2	+0.4
KHP*	4	1.266	1.272	0.1	+0.5
As₂O₃	4	1.152	1.154	0.1	+0.2
	4	2.304	2.307	0.1	+0.1

* Potassium acid phthalate.

A simple generator cell which has been employed satisfactorily for this purpose is shown in figure 1. A suitable generator electrolyte was allowed

to flow through the cell constantly during the course of the titration. The effluent solution from the appropriate delivery tube was then allowed to flow into the solution containing the sample.

Typical results which have been obtained with this technique are summarized in the table. The generator electrolyte which, on electrolysis, furnished the hydrogen and hydroxyl ions for the acid-base titrations was 1.0 M sodium sulfate and that which furnished the iodine for the arsenious oxide titration was 1.0 M potassium iodide. A constant generation current of 0.2500 amp. was maintained in all titrations. The reproducibility of the analyses is excellent. The finite time which is required for mixing apparently causes a significant delay in indicator response at the equivalence point. This delay is believed to be the primary source of the small but consistent positive error which was observed in all determinations. Automatic devices which will anticipate the approach of the end-point and thus permit greater accuracy in the determination of the generation time are now being studied in the hope of eliminating this systematic error.

Further studies on the applicability of this technique to a wide variety of titrations, including precipitation titrations, are now in progress in this Laboratory.

[1] Parrington, P. S., and Swift, E. H , *Anal Chem.*, 22, 889 (1950)   References to previous work by Swift and his coworkers are given in this paper.

[2] Cooke, W. D., and Furman, N. H., *Ibid.*, 22, 896 (1950).

A NEW INHERITED ABNORMALITY OF HUMAN HEMOGLOBIN*

BY HARVEY A. ITANO† AND JAMES V. NEEL

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In the course of a study of the inheritance of the sickling phenomenon[1] two families were encountered in which there occurred one or more children with a hematological picture which resembled that of sickle cell disease but was of less severity. The situation further differed from that usually encountered in sickle cell disease in another important respect. It has been shown that in the great majority of instances both parents of a child with

sickle cell disease exhibit the sickle cell trait.[1,3] In each of these two families, however, the erythrocytes of only one parent could be induced to sickle.

Electrophoretic studies[3,4] have demonstrated the presence of an inherited abnormality of hemoglobin in sickle cell disease and sickle cell trait. Similar studies of various members of these two anomalous families have now been carried out and have led to the recognition of a new inherited abnormality of hemoglobin. The detailed results of the electrophoretic analyses are given in table 1. In family A the hemoglobin of the propositus was found to consist of three electrophoretic components.

TABLE 1

	AGE	SICKLING TEST	PATTERN (FIG. 2)	HEMOGLOBIN COMPONENT,[a] %		
				NORMAL	SICKLE	NEW COMP.
Controls						
Normal	..	—	*a*	100	—	—
Sickle cell anemia	..	+	*b*	—	100[b]	—
Sickle cell trait	..	+	*c*	55–76	24–45[b]	—
Family A						
Father (P. C., Sr.)	29	—	*d*	64.7	—	35.3
Mother (E. M. C.)	28	+	*c*	66.5	33.5	—
Brother (P. C., Jr)	6	—	*d*	66.4	—	33.6
Brother (R. G. C.)	4	—	*a*	100	—	—
Propositus 1, ♀ (P. A. C.)	3	+	*f*	13	39	48
Family B						
Father (J. W.)	33	—	*d*	69.8	—	30.5
Mother (D. F. W.)	31	+	*c*	68.9	31.1	—
Propositus 2, ♀ (R. W.)	12	+	*e*	—	47	53
Propositus 3, ♂ (T. W.)	10	+	*e*	—	50	50
Brother	8	—	*a*	100	—	—

[a] The authors are indebted to Dr. Ibert C. Wells for carrying out these computations.
[b] Wells, I. C., and Itano, H. A., in press. In some cases the hemoglobin of individuals with the clinical diagnosis of sickle cell anemia contains a small fraction (5 to 20 per cent) of normal hemoglobin.

The mobilities of two of the components correspond to those of the hemoglobins from normal and sickle cell anemic individuals, respectively. The other component, which migrates as a more positive ion than either normal or sickle cell anemia hemoglobin, has hitherto not been encountered. In family B the hemoglobin of each of the two propositi was found to be a mixture of two types, namely, that characteristic of sickle cell disease and the new fraction. Electrophoretic studies of the remaining members of the families revealed that in each family the hemoglobin of the parent whose erythrocytes sickled exhibited the electrophoretic findings typical of sickle cell trait,[4,5] while the hemoglobin of the other (non-sickling) parent was a mixture of two types, normal and the new component detected in the pro-

positi.   The families involved in the study are pictured in figure 1.   The propositi through whom the study was initiated are indicated by arrows.

Carbonmonoxyhemoglobin solutions were prepared and analyzed electrophoretically in the Tiselius apparatus; exact details of the preparation and analysis have recently been reported.[5]   The carbonmonoxyhemoglobin solutions were diluted to 1.0 per cent concentration and dialyzed against cacodylate-sodium chloride buffer[6] of ionic strength 0.1 and pH 6.50 for eighteen hours.   The final scanning diagrams were taken after fifteen hours of electrophoresis at 1.5°C. at a potential gradient of 3.49 to 3.60 volts per centimeter.   The mobilities in the ascending boundaries of the carbonmonoxyhemoglobins from normal and sickle cell anemic individuals
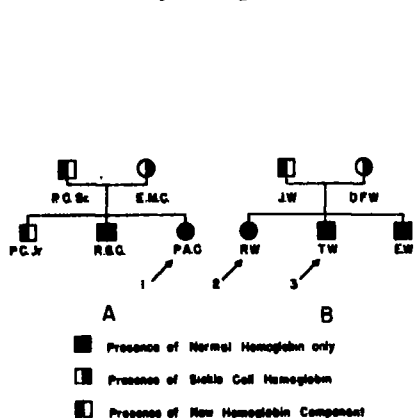
FIGURE 1

The two families under study.
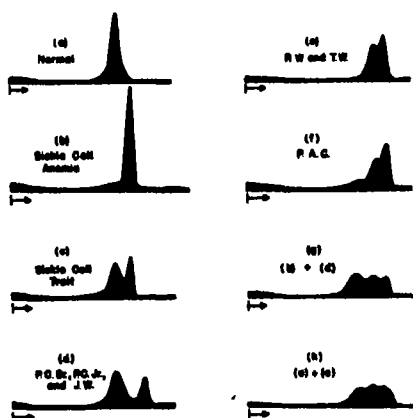
FIGURE 2

The Longsworth scanning diagrams of the carbonmonoxyhemoglobins of the individuals under study compared to the scanning diagrams of the carbonmonoxyhemoglobins of individuals known to be hematologically normal or to have sickle cell anemia or sickle cell trait.

under these conditions are 2.4×10^{-5} and 2.9×10^{-5} cm./sec. per volt/cm., respectively.   The mobility of the new component is 3.2×10^{-5} cm./sec. per volt/cm.

The Longsworth scanning diagrams of the ascending boundaries are shown in figure 2.   The various components are visible in the scanning diagrams of the descending boundaries, but the resolution of the peaks is poor.[5]   Patterns (*a*) and (*b*) are those of the hemoglobins from normal and sickle cell anemic individuals, respectively, obtained under the experimental conditions described above.   The hemoglobins of two of the siblings (R. G. C. and E. W.) of the propositi showed the normal pattern.   Pattern (*c*), a typical sickle trait pattern containing both the normal and sickle cell

anemia hemoglobins, is similar to those of the hemoglobins of the sickling parents (E. M. C. and D. F. W.) in this study. Pattern (*d*) shows two components, normal hemoglobin and the new component, but lacks the sickle cell anemia component. This pattern was found in both the non-sickling parents (P. C., Sr., and J. W.) and in a brother (P. C., Jr.) of one of the propositi. The hemoglobins of two of the propositi (R. W. and T. W.) gave the two-component pattern, (*e*), which appears to contain sickle cell anemia hemoglobin and the new component but not normal hemoglobin. The hemoglobin of the third propositus (P. A. C.) resolved into all three components, as shown in pattern (*f*). The analysis of a solution prepared by the addition of one part of sickle cell anemia hemoglobin to two parts of P. C., Sr.'s hemoglobin resulted in pattern (*g*), which clearly shows three components. A similar result, pattern (*h*), was obtained by the addition of one part of normal hemoglobin to two parts of R. W.'s hemoglobin.

The foregoing results lead to the conclusion that a previously unreported protein component, differing in electrophoretic mobility from the hemoglobins of normal and sickle cell anemic individuals, is present in considerable amounts in the erythrocytes of certain individuals. Other observations indicate that this component is indeed another abnormal hemoglobin. The presence in the erythrocytes of a protein other than hemoglobin to the extent of from one-third to one-half of the total protein contents of the cells would cause a markedly low mean corpuscular hemoglobin concentration[6] instead of the normal values which have been observed. Dilution of the different hemoglobin preparations to 1.0 per cent concentration was based on a spectrophotometric determination using a standard curve obtained at 540 mμ with normal hemoglobin.[5] Subsequent electrophoretic analyses of these diluted solutions revealed that the total area of the scanning diagram was in each case equal to that obtained with a 1.0 per cent solution of normal hemoglobin. This indicates that within the error of this method the molecular extinction coefficient at 540 mμ is the same for all three components, assuming equal molecular weights. It may be noted that the color of the fast moving component in the ascending boundary was observed to be the same as that of normal carbonmonoxyhemoglobin.

The data which have been presented suggest that the tendency to form the new hemoglobin component is inherited as if due to a single dominant gene. The effect of this gene in the homozygous condition is not yet known; it is possible that it corresponds to some already recognized hematological syndrome. The relationship of this gene to the sickle cell gene is not clear at the present time. The hematological picture in the individuals who may be postulated to have received both genes is explicable either on the basis of the factor interaction on the part of two independent genes, or as a consequence of multiple allelism.

A detailed hematological delineation of this new entity is in progress. Physicochemical investigations to further characterize the new hemoglobin component are also in progress.

The assistance of the Hematology Clinic of the Children's Hospital of Detroit in many different aspects of these studies is gratefully acknowledged.

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⁴ Pauling, L., Itano, H. A., Singer, S. J., and Wells, I. C., Science, 110, 543 (1949).
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⁶ Perutz, M. F., Nature, 161, 204 (1948).

BIOELECTRIC POTENTIALS AS A MEASURE OF RADIATION INJURY

BY A. A. BLESS

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The criteria used to measure the biological effects of radiation by most workers as well as by the writer are in a sense purely biological in character, such as the size of the plant or embryo, the number of surviving colonies and the like. In 1942, Professor A. L. Romanoff of Cornell University worked with the writer on the effects of x-rays on the diameter of the developing blastoderm of the chick egg and also on the effects of x-rays on the bioelectric potential of the blastoderm.[1,2] Dr. Romanoff and the writer were greatly impressed by the similarity of the curves representing the variation of the two parameters with dosage. The experiments seemed to indicate that the bioelectric potentials may be as reliable a measure of the effectiveness of radiation as the size of the embryo.

Because of the War, work was not resumed in this field until recently. In 1947 Jones[3] measured the variation of the bioelectric potential of seeds of corn and beans with x-ray dosage. The shape of the curves was exponential, typical of the variation of many relevant parameters with dosage. He also confirmed the results of Nelson and Burr[4] that the more vigorous strains of seeds have a higher potential. Since radiation affects the vigor of a plant, these results again indicated that potentials may be used as a measure of the effectiveness of radiation. Hunter[5] using wheat seedlings, which have easily measurable coleoptiles, found in some of his

measurements a similarity between the curves representing the variation of the length of coleoptile with x-ray dosage and the variation of the potential of the seed with the same dosage.

It seems worth while to examine carefully the relationship between the potential measurements and radiation injury, and the extent of validity of this relationship. Wheat seedlings were used in these experiments because of their conveniently measurable coleoptile lengths with which potential measurements may be compared.

The work of Carleson[6] and others established the fact that x-rays are most effective when the cells are in a certain stage of mitosis. It was of
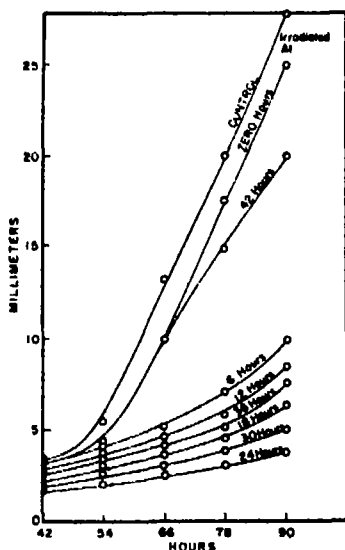
FIGURE 1

The length of coleoptiles as a function of time for seedlings given the same dose at different stages of growth.
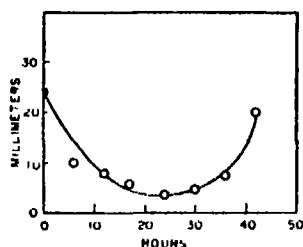
FIGURE 2

The length of coleoptiles at the same age as a function of time at which the radiation was applied.

interest in this work first to find the age of the seed at which most of the cells are in this stage, or the age most favorable for irradiation at room temperature. For this purpose groups of 30 seeds each were subjected to the same radiation dosage, 3000 r units, at 0, 6, 12, 18, 24, 30, 36, and 42 hrs. after immersion in nutrient solution. The lengths of coleoptiles were measured shortly after these appeared (about 48 hrs. after immersion in nutrient solution) and at 12-hr. intervals thereafter. Figure 1 shows the variation of the length of coleoptile as a function of time for each exposure. The effectiveness of radiation varies with the age at which the dose is administered, as was to be expected and is most easily shown by plotting the

length of coleoptile at a given stage of growth against the age at the time of exposure. Figure 2 shows such a curve for which the length measurements were made at 96 hrs. It is evident that radiation at 24 hrs. is most effective. Since the curves of figure 1 do not intersect, the same age for maximum effectiveness would have been obtained if the curve of figure 2 had been drawn for any other age after 72 hrs. However, the actual effectiveness (fractional length reduction) would have been different. It must be emphasized that this age for maximum effectiveness holds only for the room temperature of our laboratory (about 25°C.). The development of the seeds can be accelerated or retarded by a higher or lower temperature, and at a different temperature the radiation would be most effective at a different age.

Measurements of potential difference were made with an electrometer tube circuit. They were taken from the tip of the coleoptile to the seed
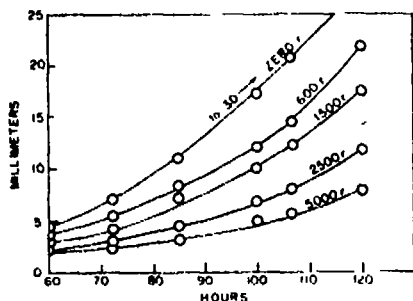
FIGURE 3

The length of coleoptiles of seeds subjected to various radiation doses as a function of time. Radiation administered at age of 24 hours.
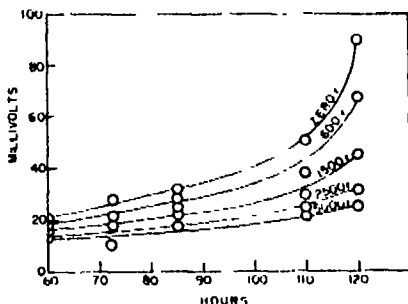
FIGURE 4

Potential of seeds subjected to various radiation doses as a function of time. Radiation administered at age of 24 hours.

coat. The experimental procedure is given elsewhere,[7] and will not be repeated here.

Figure 3 shows a series of curves of the length of coleoptile as a function of time of growth for several different radiation doses, all administered 24 hrs. after immersion in nutrient solution. Every point of the data presented here is the average of at least 30 seeds. For one reason or another, much of the data was retaken and many of the points on the curves shown represent an average of 60 seeds or more. Figure 4 gives the potential as a function of time for the same seeds. The most striking feature is the similarity of the two sets of curves, and both of them show clearly the diminution of the parameter with increasing dosage. The diminution of the two parameters is not in the same ratio. For example, at 72 hrs. the ratio of the length of coleoptile of control to that of highest exposure is 2 while at

120 it is 4. The ratio of potentials for the two periods is $1^1/_2$ and $3^1/_2$, respectively.

The effects of radiation on each of the two parameters is perhaps better shown in figures 5 and 6 where the length of coleoptile and the magnitude of the potential at a given stage of growth are plotted as a function of radiation.

There were a number of differences in the data on the measurement of the lengths of the coleoptiles and of the potentials which must be noted here. In the first place, some seeds showed a reversal of polarity, i.e., the coleoptile was positive with respect to the seed coat instead of being negative as in the great majority of the cases. The averages presented in figures 3–6 were obtained without regard to sign. The exclusion of the cases of opposite polarity would have affected the average of each group only
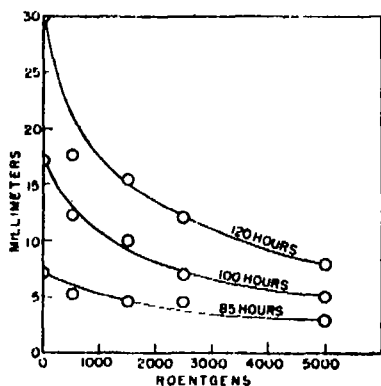
FIGURE 5

The length of coleoptiles at a given age as a function of dosage.
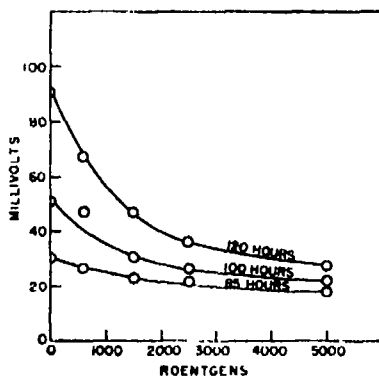
FIGURE 6

The potentials of seedlings at a given age as a function of dosage.

slightly and would have made little difference in the shape of the curve. It is interesting to note that the number of cases of opposite polarity decrease with increasing dose, being completely absent in groups subjected to heavy radiation doses.

Another extremely important difference is in the distribution of the magnitudes of each parameter within a given group. The lengths of coleoptiles of a given group are much more homogeneous than corresponding potential measurements, all length measurements being crowded close to the average. In each group there were a few length measurements which differed considerably from the average, but these few were clearly exceptions (giants or dwarfs; in the majority of cases dwarfs). If these are excluded, the range of variations within a given group is rarely greater than threefold.

This uniformity is not true at all for potentials. The individual values are much wider apart, a 20-fold variation within a given group not being uncommon, and all values seem to be represented. The exclusion of readings having a deviation four times the average deviation for a given group did not materially affect the average value of the potentials of the group. Figures 7 (*a*) and (*b*) show the distribution of coleoptile lengths for control and for a 3000 r dose, respectively, while (*c*) and (*d*) show the distribution of potentials for the same two groups. The variation in distribution of values of the two parameters is evident. It was noticed that the range of variation of values of coleoptiles or potentials were much smaller with increasing radiation dose. This is also demonstrated in figure 7.

The difference in distribution of the values of the lengths of coleoptiles
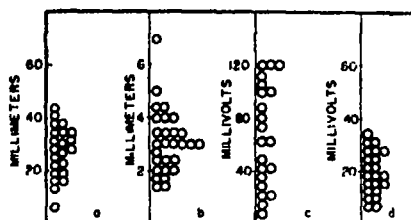
FIGURE 7

Distribution of (*a*) length of coleoptiles, control; (*b*) length of coleoptiles subjected to a dose of 3000 r; (*c*) potentials, control; (*d*) potentials of seeds subjected to a dose of 3000 r.
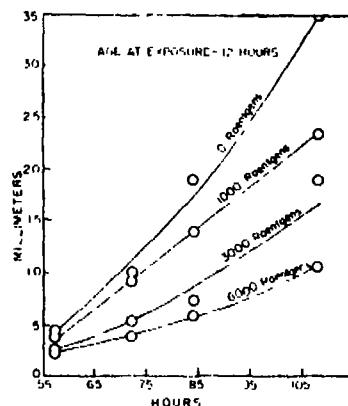
FIGURE 8

The length of coleoptiles of seeds subjected to various radiation doses as a function of time. Radiation administered at age of 12 hours.

and the magnitudes of potentials is to be expected. The length of the coleoptile is the sum total of cell activity over a considerable period of time. The larger the period the smaller is the range of variation of that parameter because individual differences seem to be ironed out. This is not true at all in the case of potentials. These measure the cell activity at a period immediately preceding the measurement or at the very time of measurement. Cell activity at a given time may vary widely, thus giving widely different values of the potential. A confirmation of this view was found in the fact that while with some seeds the galvanometer showed a steady deflection, with others the deflection did not remain steady at all, and fluctuated slightly. Another confirmation of this view was found in the fact that seeds with large coleoptiles did not necessarily give large values

of the potential. The cell activity even in a very vigorous seed may be small during a given time interval, thus giving small potential immediately after or during these intervals.

The large variation in the values of potentials may lead to erroneous conclusions if only a small number of observations are used. Figure 8 shows the variation of the coleoptile length of seeds subjected to different radiation doses 12 hrs. after immersion in nutrient solution, while figure 9 shows the values of the potentials of the same seeds. Figure 8 is very similar to figure 3, which shows the variations of coleoptile length with age at various exposures 24 hrs. after immersion in nutrient solution. On the other hand the first two curves of figure 9 are not at all similar to those of figure 4, being convex upward instead of concave. These curves would
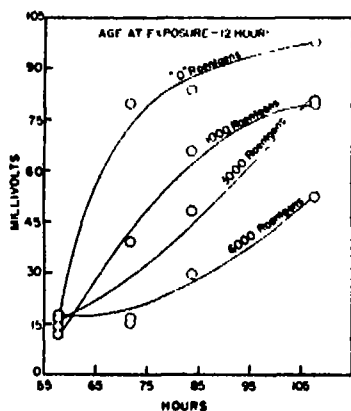
FIGURE 9

Potential of seeds subjected to various radiation doses as a function of time. Radiation administered at age of 12 hours.
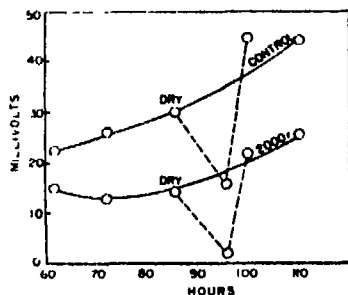
FIGURE 10

The potential of seeds subjected to dryness as a function of time.

give ratios of effectiveness of radiation much larger than those of coleoptile length and different from those for potentials mentioned earlier.

However, these two curves are truly exceptions, since out of some 40 or more sets of readings obtained in the course of the investigation these were the only ones which were so much out of line with the rest. On the basis of probability, such curves are to be expected if the number of specimens used is small, illustrating the danger of making conclusions from potential measurements of a few specimens. This naturally limits the usefulness of potential measurements for measuring radiation injury. On the other hand bioelectric potentials are very sensitive to temporary changes in the condition or environment of the organism. A lucky mishap brings this out very clearly. Because of neglect a tray containing groups

of seeds subjected to various radiation doses was not watered at a certain period of growth. Readings of the lengths of coleoptiles and of the values of the potentials were continued after the accident was noted, and after the tray was watered. The lengths of coleoptiles measured at the end of the "dry" period showed no appreciable departure from the curve. However, the potential showed a violent departure. Figure 10 shows the potential for only two of the groups of seeds, control and 2500 r exposure, as a function of time in the nutrient solution, the curves for three other exposures following exactly the same pattern. It is evident that the potentials suffered a very large drop.

It is reasonably safe to say that the average value of the potential of specimens is as good as measure of radiation injury as any other parameter, providing an adequate number of specimens are used. It is possible that a series of readings on one specimen may give as good an indication of radiation injury as a single reading of many specimens. Work is now being done on this problem. Work is also in progress on the length of time between the application of the radiation stimulus and the bioelectric response.

Thanks are due to B. Kivel and A. Rosenberg for their help in taking these data.

This work was done under the auspices of the Atomic Energy Commission.

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THE LOCI OF ACTION OF ULTRA-VIOLET AND X-RADIATION, AND OF PHOTORECOVERY, IN THE EGG AND SPERM OF THE SEA URCHIN

BY HAROLD F. BLUM,* J. COURTLAND ROBINSON AND GORDON M. LOOS

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Given in moderate doses, both ultra-violet radiation and x-ray delay cell division (cleavage) of the eggs of the sea urchin *Arbacia punctulata*. The present communication summarizes experiments which indicate that the locus of this action is, in both cases, associated with cell nuclei, as contrasted to cell cytoplasm. There is recovery from the effect of the radia-

tion, as indicated by return toward the normal rate of cleavage. Following treatment with ultra-violet radiation, recovery is accelerated by illumination with "visible" radiation. Our experiments indicate that this photo-recovery has its locus in the cytoplasm of the egg. In the case of x-ray, visible radiation appears to be without effect on the recovery of cleavage rate.

We are concerned here with the conclusions that may be reached by summarizing the results of a variety of experiments. Some of these have already been discussed in other papers, which include descriptions of the general method used.[1] Those results which have been newly added will be described in greater detail elsewhere.

Locus of the Delay of Cleavage by Ultra-Violet Radiation.—Advantage has been taken of the fact that the eggs of *Arbacia* can be separated by centrifugation into nucleate and enucleate halves, adding to the number of experimental combinations with which one may work. The whole eggs may be exposed to ultra-violet radiation,[2] either before or after fertilization with normal sperm. The same procedure may be followed with the

PART EXPOSED TO ULTRAVIOLET	☉	☉☉	☉	☉	☉	☉	—	—	—
	+		+		+		+	+	+
NORMAL PART	—		—		—		☉	☉	○
DELAY	+	+	+	+	0	+	+	+	+

		unfertilized	fertilized	
CODE	whole egg	☉	☉☉	sperm
	nucleate half	☉	☉☉	—
	enucleate half	☉	☉	

FIGURE 1

nucleate or enucleate halves. Or, the sperm may be exposed to the ultra-violet radiation before it is used to fertilize the eggs or halves. Various combinations are indicated in figure 1, which also summarizes the results with regard to the delay of cleavage. Delay is observed in all cases except when the enucleate half is exposed to ultra-violet radiation before fertilization with normal sperm. This is also the only case in which the part that receives the radiation contains no nucleus. If the sperm nucleus is introduced into the enucleate half by fertilization before the exposure, or if the sperm itself is exposed to the ultra-violet radiation, delay of cleavage results. The conclusion seems obvious that the locus of action of the radiation is the nucleus or something closely associated with it. Reasons will be discussed shortly for thinking that nucleoprotein is the substance absorbing the ultra-violet radiation in the primary photochemical act leading to delay of cleavage.

Locus of Photorecovery.—After the initial delay by ultra-violet radiation, there is a gradual return toward the normal cleavage rate. This recovery

process is greatly accelerated by illumination with "visible" radiation.[3] To determine the locus of the photorecovery process, experimental combinations similar to those already described were employed. The results are summarized in figure 2. In the case of the enucleate half exposed to ultra-violet before fertilization, the presence or absence of photorecovery cannot be demonstrated because there is no delay of cleavage. Photorecovery was observed in all other cases except that of the sperm before introduction into the egg. In such experiments, a sample of sperm was exposed to ultra-violet radiation, and then divided into two portions, one of

PART EXPOSED TO ULTRAVIOLET										
PART ILLUMINATED WITH 'VISIBLE'										
PHOTORECOVERY	+	+	+	+	+		+	+	+	+	0

FIGURE 2

which was illuminated with visible radiation, while the other was placed in the dark. One hour later, samples of normal eggs were fertilized, one with the illuminated sperm, the other with the sperm that had been kept in the dark. The two samples of eggs underwent cleavage at the same time. On the other hand, when sperm which had been exposed to ultra-violet radiation were used to fertilize normal eggs, photorecovery was observed. That is, those fertilized eggs subsequently illuminated with visible radiation recovered more rapidly than those kept in darkness. Nucleate or enucleate halves may take the place of the whole eggs in this experiment. Thus, it

PART EXPOSED TO X-RAY							
	+		+		+		+
NORMAL PART	—		—		—		
DELAY	+	+	+	+	0	+	+
PHOTORECOVERY		0					0

FIGURE 3

appears that egg cytoplasm is essential for the photorecovery process.

The sperm lacks altogether the power to recover, whether in light or darkness. If two samples of the same preparation of sperm are exposed to doses of ultra-violet radiation identical in amount, but applied one hour later in the one case than in the other, there is no difference in the resultant amount of cleavage delay when normal eggs are fertilized with these samples of sperm. Thus, the ability to recover, whether in light or dark, is associated only with egg cytoplasm.

Locus of Delay of Cleavage by X-Ray.—Experiments with x-ray are summarized in figure 3. The results parallel those with ultra-violet radiation

in showing that the effect is associated with the nucleus. In contrast, however, visible radiation was not found to accelerate recovery from x-ray, indicating that this agent acts in a fundamentally different way from ultra-violet radiation. Figure 3 has been amplified by including, together with our own results, some of those reported by Henshaw. Henshaw's extensive experiments on unfertilized eggs[4] we have not repeated.[5] He found, as did we, that when exposed to x-ray before fertilization, nucleate halves showed delay of cleavage, but enucleate halves did not.[6] He mentions none of the other experimental combinations with nucleate and enucleate halves, which we report. Henshaw found, parallel to our experiments with ultra-violet, that sperm exposed to x-ray showed no power of recovery.[6]

Discussion.—The photorecovery after ultra-violet radiation, manifested by the *Arbacia* egg, seems in all ways parallel to the "photoreactivation" in fungi and bacteria, which has been the subject of a number of papers since it was reported by Kelner[7] a year ago. The phenomenon has been demonstrated in widely separated animal and plant forms.[8] The experiments described herein seem to parallel closely those of Dulbecco[9] with bacteriophage and *Escherichia coli*, so special reference will be made to that work. The wave-lengths effective in photorecovery and photoreactivation are the same. In both cases, the visible light has no demonstrable effect unless some part of the system has been previously exposed to ultra-violet radiation.[1, 9] Indeed, these aspects are characteristic of the phenomenon wherever it has been studied. If the sperm is taken to be comparable to bacteriophage, and the egg to be comparable to *E. coli*, the parallelism is revealed. Sperm, like bacteriophage, does not show recovery from the effect of ultra-violet radiation. Only when the irradiated sperm or bacteriophage is associated with egg cytoplasm or *E. coli*, respectively, does recovery take place, and it is only under these conditions that light plays a role in accelerating recovery. *E. coli* itself, like the *Arbacia* egg, shows photorecovery from the effects of the ultra-violet radiation.

Since the bacteriophage is virtually pure nucleoprotein, it seems almost certain that this substance is the absorber of the ultra-violet radiation. It is also reasonable to think that nucleoprotein of the nucleus is again the absorber of ultra-violet radiation in the primary photochemical act initiating cleavage delay. It is important to point out that our experiments do not preclude action of ultra-violet radiation on parts of the cell other than the nucleus, but only indicate that such action does not affect the rate of cleavage of the egg.

Neither sperm nor bacteriophage can "repair" the "damage" done by ultra-violet radiation, this being a function of egg cytoplasm or *E. coli*, respectively. Moreover, neither the sperm nor bacteriophage is strictly a self-reproducing system. The bacteriophage multiplies only in association

with the host cell, which is presumably essential for synthesis of nucleoprotein. Similarly, the sperm nucleus is associated with cell division and the synthesis of nucleoprotein only after it is brought into the presence of egg cytoplasm. In attempting to explain this parallelism, let us postulate that ultra-violet radiation alters the nucleoprotein, bringing about some minor change in configuration that can be reversed by the synthetic processes carried out by systems generally present in the cytoplasm,[10] but absent in the case of the sperm. It is tempting to associate the repair after exposure to ultra-violet radiation with the synthesis of nucleoprotein; and since the latter no doubt involves endergonic processes, to regard the repair, too, as endergonic. It is tempting, further, to think that visible radiation accelerates the repair by contributing energy to this endergonic process. But this may be carrying speculation beyond the point of profit, unless further evidence can be supplied.

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THE IMMEDIATE DEPENDENCE OF THE ACTION OF A SPECIFIC GENE IN DROSOPHILA MELANOGASTER UPON FERTILIZATION

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In *Drosophila melanogaster*, a particular gene complex composed of a semidominant mutant, erupt (*er*), together with a semidominant suppressor of erupt (*Su-er*), was discovered by the senior author[1] and found to be widely distributed in laboratory stocks and wild populations of this

species.[2] (The erupt phenotype consists of a large eruption of non-faceted material usually through the center of one or both eyes. Sometimes this structure bears bristles. In weaker manifestations of erupt the facets in the central region of the eye are merely disarranged. There may be a small extra bristle on the anterior margin of the eye, dorsal to the antenna.) The two loci are in separate linkage groups and segregate in characteristic Mendelian fashion. The loci are demonstrably not strictly duplicate loci, for the two reciprocal genotypes $Su\text{-}er^+$ $Su\text{-}er^+$; er^+ er^+ and $Su\text{-}er$ $Su\text{-}er$;

TABLE 1

THE EFFECTS OF X-RAYS APPLIED AT VARIOUS AGES IN BLOCKING THE ACTION OF THE SUPPRESSOR-ERUPT GENE

AGE AT RADIATION (HRS. POST-LAYING)	TOTAL EGGS	TOTAL ADULTS ENCLOSED AND CLASSIFIED	EYE PHENOTYPE			ERUPT, %		DIFFERENCE AND S. E. DIFF.
			NORMAL	WEAK ERUPT	EXTREME ERUPT	TOTAL	EXTREME	
1 → 0.25	1973	63	2	7	54	96.8	85.7	
Control	491	424	413	10	1	2.6	0.24	0.94 ± 0.05
3 → 0.5 (a)	1066	106	40	38	28	62.3	26.4	
Control (a)	165	158	123	34	1	22.2	0.6	0.40 ± 0.06
3 → 0.5 (b)	2083	41	4	7	30	90.2	73.2	
Control (b)	260	241	233	8	0	3.3	0.0	0.87 ± 0.06
5 → 1	1063	34	0	9	25	100.0	73.5	
Control	323	276	274	2	0	0.73	0.0	0.99 ± 0.06
10 → 1	310	47	2	14	31	95.7	65.9	
Control	150+	150	149	1	0	0.7	0.0	0.95 ± 0.07
15 → 1	529	36	0	5	31	100.0	86.1	0.78 ± 0 09
Control same as for 3 → 0.5 (a)								
18 → 1	1488	225	27	50	148	88.0	65.8	
Control	255	232	223	9	0	3.9	0.0	0.84 ± 0.05
24 → 1 (Larvae)	342	147	2	13	132	98.6	89.8	
Control	260	246	246	0	0	0.0	0.0	0.99 ± 0.05
LARVAE (HRS. POST-HATCHING)								
55	292	217	38	28	151	82.5	69.6	
Control	390	359	355	4	0	1.1	0.0	0.82 ± 0.04
75	175	160	115?	7?	38	28.1	23.8	
Control	235	209	202	7	0	3.4	0.0	0.25 ± 0.04
100	140	120	113?	7?	0	5.8	0.0	0.025 ± 0.02

er er, which would be identical if the two loci were duplicates ($Su\text{-}er$ being equivalent to er^+ and $Su\text{-}er^+$ to er), do not respond alike to a certain specific environmental agent. If embryos at an age of 10 hours are given an x-ray dose of 1000 r units, the eyes of the enclosing adult flies of the first genotype given above are quite normal, whereas those of the second genotype express a strong manifestation of the erupt character in from 90 to 100% of the treated individuals. The effect of x-rays on the particular genotype $Su\text{-}er$ $Su\text{-}er$; er er was attributed to a specific blocking of some phase of the action of the suppressor gene.

In order to determine the critical period during which an x-ray treatment will inactivate the effect of this suppressor-erupt gene, doses of 1000 r units were applied to embryos aged 1 hour ± ¹/₄ hour, 3 hours ± ¹/₂ hour, 5 hours ± 1 hour, 10 hours ± 1 hour, 15 hours ± 1 hour, 18 hours ± 1 hour, and 24 hours ± 1 hour. These treatments covered the entire embryonic period from 1 hour after the egg is laid to a time when the eggs have hatched into first instar larvae. All cultures were kept at 25 ± 1°C. As table 1 and figure 1 show, the inactivation of the suppressor-erupt effect was indistinguishable at all of these ages from that exerted upon embryos 10 hours old and was virtually complete.
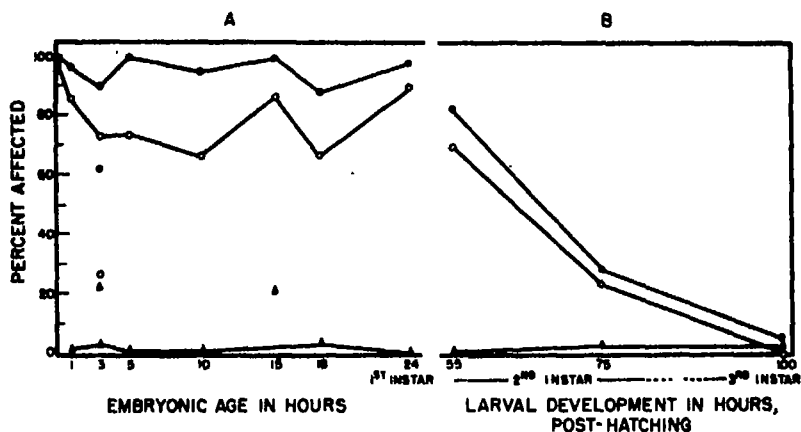
FIGURE 1

Percentages of individuals manifesting erupt eyes when treated with 1000 r units of x-rays at various ages. ●, per cent manifesting erupt to any degree, x-rayed series; O, per cent manifesting extreme erupt, x-rayed series; ▲, per cent manifesting erupt to any degree, untreated control series.

Controls in each case are from eggs laid by the same parents who laid the eggs treated with x-rays. All control series are homogeneous except for series (a) at 3 ± .5 hours. In this series, the percentage of weak erupt was very high, and simultaneously the inactivation of the suppressor in the treated individuals was far less complete than usual, although the control and treated series were still significantly different. The test at this age was therefore repeated [series (b)] with results quite in line with those obtained in all the other series. In figure 1, the values obtained in series (a) at 3 hours are plotted, but have not been used in drawing the curves.

Extension of the tests into the larval period showed that the inactivation of the suppressor-erupt effect is still nearly maximal 55 hours after the larvae have hatched from the egg, at a time well into the second larval instar. At 75 hours of larval development, in the third instar, the effectiveness of the x-ray dose has diminished very considerably, and by 100 hours of larval age it has almost completely disappeared.

Disarrangement of the facets is so general in individuals treated with x-rays at 75 or 100 hours of larval life that classification of weak erupt could only be based on the presence of the extra bristle on the anterior dorsal margin of the eye. The (?) signs in table 1 in these two series indicate this inability to classify erupt on the same basis as in the other series.

The effectiveness of the x-ray treatment so very early in the embryonic period led us to make a further effort to obtain and treat eggs as young as they could possibly be collected in sufficient numbers and also to see whether the treatment of the spermatozoa and oöcytes prior to fertilization might be effective. It proved possible to collect and treat eggs at an average age of 8 minutes ± 8 minutes after they were laid. Care was taken to collect eggs only from females laying actively and, by discarding the first collection made, to eliminate any eggs which might have been stored in the female after having been fertilized. The x-ray treatment (see table 2) proved just as effective in eliminating the suppression of erupt in these

TABLE 2

THE EFFECTS OF X-RAYS ON THE SUPPRESSOR OF ERUPT PRIOR TO AND SUBSEQUENT TO FERTILIZATION

		PHENOTYPE OF EYES			ERUPT, %	
A. X-RAYED 1000 r	TOTAL CLASSIFIED	NORMAL	WEAK ERUPT	EXTREME ERUPT	TOTAL	EXTREME
Adult females	339	335	4	0	1.18	0.0
Adult males	279	277	2	0	0.72	0.0
Adult males and females	406	402	4	0	0.99	0.0
Control (not x-rayed)	440	436	4	0	0.91	0.0

$$[\chi^2 = 0.364 \qquad n' = 3 \qquad P = 0.95 - 0.90]$$

B. X-RAYED 1000 r						
Embryos: 8 ± 8 minutes	49	0	1	48	100.0	98.0
Control (not x-rayed)	227	226	1	0	0.44	0.0

newly laid eggs as it had in older embryos, 98% of the flies hatching from them manifesting strongly erupt eyes. On the contrary, neither radiation of spermatozoa nor of oöcytes separately, nor of both together, produced any inhibition of the suppressor effect. This was true even when the dosage of x-rays was increased to 2500 r units. In these first treatments of the germ cells before fertilization, the treatments were given separately to mature males and females, which were thereafter mated. In order to shorten the time between treatment and fertilization, we next treated with x-rays females which had already been inseminated. Such females would carry, at the time of treatment, one or two already fertilized eggs in utero, but most of the eggs laid would come from oöcytes and spermatozoa treated prior to fertilization. As expected, a very few erupt flies were produced, and in cultures from single females these were distributed as follows: 16 with no erupt offspring; 6 with 1 erupt; 2 with 2 erupt; 1 with 3 erupt. The overwhelming majority of offspring showed complete suppression of erupt.

The difference between the two series, the one treated prior to fertilization and the other as soon as possible subsequent to it, is very striking and statistically significant far below the 0.001 level ($\chi^2 = 980.7$; $n' = 2$). Since, according to the studies of Huettner,[3] meiosis of the egg is arrested at metaphase of the first meiotic division until fertilization occurs, the completion of meiosis and the formation of the polar bodies must occur within the 8 minutes before the fertilized eggs were treated. According to Rabinowitz,[4] the fertilized egg of Drosophila takes 23 ± 1.72 minutes at 24°C. to reach the first division of the cleavage nuclei, and at 15 ± 1.21 minutes it is either in telophase of the second maturation division or at the conjugation of the pronuclei. It therefore appears that the 8-minute-old embryos treated in this experiment could hardly have reached the first cleavage division, on the average. This appears to signify that as the entry of the sperm activates the egg to form its polar bodies, so too it activates it to produce at least one specific gene-initiated substance or morphogenetic system, namely, that one which is due to the presence of the suppressor-erupt gene and which is inactivated by x-rays. Still, this specific reaction that follows immediately upon activation of an insect egg by fertilization is far from being obviously related to such activation phenomena as an increased rate of respiration, production of acid, superficial cytolysis, release of calcium ion, etc., which occur in echinoderm eggs upon fertilization (see reviews by Lindahl[5] and Tyler[6]). One may suppose, however, that the action of the suppressor-erupt gene may, according to the theory of Runnström,[7] involve a more intimate contact between catalysts and substrate or the liberation of a mediator. Isolation of the chemical system which is inactivated by x-rays would clarify this problem. Meanwhile it remains uncertain whether the postulated inactivated substance is to be regarded as enzyme or substrate.

To our knowledge, this is the most precise determination yet made of the time at which the action of a specific gene is determined. The reasoning upon which this is based is as follows: (1) Production of the complete blocking effect by exposure to x-rays at any age up to 24 hours, by which time there are great numbers of nuclei, and furthermore the failure of the blocking effect to be transmitted through the germ line to the next generation, alike indicate that the effect of the x-rays is not at all to cause the suppressor gene itself to mutate, but is rather to inactivate some product of this gene. The action of the x-rays is direct and cannot in some way be stored so as to produce a later effect on a substance or morphogenetic system that was not present at the time of irradiation. This follows from the fact that treatment of mature oöcytes and spermatozoa is completely ineffective, whereas the treatment of just fertilized eggs is completely effective. There is absolutely no carry-over. Moreover, the target altered in nature by the irradiation is probably to be identified as a product of the

suppressor gene, for it is not present in the absence of the suppressor gene, i.e., in the genotype *Su-er*+ *Su-er*+; *er*+ *er*+. It could however be an essential substrate for the suppressor gene. (2) The complete blocking of the suppressor action at any age from 8 minutes up to 24 hours shows that the x-rays destroy some substance or morphogenetic system which cannot be replenished or repaired. The simplest explanation is that a specific gene product or substrate is formed in full amount prior to the earliest time at which complete inactivation can be obtained and that it persists unmodified in amount for so long a time as it is still possible to obtain complete inactivation by means of the x-rays. In other words, the gene product or substrate here involved is present in full amount within 8 minutes after fertilization and persists unchanged in amount until some time after the larva has hatched from the egg. (3) During the second and third larval instars the inhibitory effect of x-rays on the action of the suppressor of erupt diminishes progressively. This indicates either that the primary gene product or substrate is gradually being used up or that the morphogenetic system in which the product (or substrate) participates has advanced beyond the stage at which the product or substrate can modify it.

It might be supposed that the suppressor gene exerts its primary effect before the egg is fertilized but that its product is not susceptible to x-rays until after it has been in some way changed through the activation of the egg by fertilization. To clear up this question, females carrying the mutant *er* but not its suppressor (*Su-er*+/*Su-er*+; *er*/*er*) were x-rayed with the same dose used previously and then crossed with males of the genotype *Su-er*/*Su-er*; *er*/*er*. The flies obtained (*n* = 839) were not significantly different from the controls in the percentage of erupt. This proves that the suppressor gene acts subsequent to its introduction by fertilization.

It might also be questioned whether the suppressor gene acts as promptly after fertilization when it is introduced through the spermatozoon as when it is already present in the oöcyte. Experiments to determine this point are now under way. Preliminary data show that it has already produced its primary x-ray-sensitive effect by the time the embryo is 5 hours ± 1 hour old.

The fact that a specific reaction system which affects the conformation of the eye can be detected a few minutes after fertilization, although completely lacking prior to that event, makes it plausible to describe this determination of the initiation of the action of a specific gene as an instance of a *primary* gene action. It belongs in a different category than those determinations of the sensitive periods during which temperature or other modifying agents affect certain gene-controlled processes or produce phenocopies. As Goldschmidt[3] has emphasized, such sensitive periods characteristically occur during the middle to late larval and pupal periods,

at a time when the affected structures are differentiating from their anlagen (pp. 3–51). Steinberg,[9] Vogt,[10–13] Begg and Sang,[14] and others have shown, moreover, that such a sensitive or effective period by no means necessarily corresponds to the time of action of the gene. Different effects of the same gene may even have different temperature-sensitive periods. The specific gene action demonstrated here, however, not only occurs long before even the imaginal disks which give rise to the eyes are laid down but even before cleavage has progressed beyond a single division.

Indications of the precise time of action of certain genes have generally been seen in cases of genetic mosaicism, both in plants and in animals. Such situations differ in an essential respect, however, from that analyzed in the present case. The production in an organism of adjacent, sharply bounded areas contrasting in phenotype is dependent upon the fact that the individual was originally heterozygous (or mosaic) as a whole, or has become heterozygous through mutation in some part of its body. What is actually observed in the mosaic is the product of an event giving rise to a segregation of alleles and consequently to a difference of genotype in particular cell lineages. It is this event which is precisely determinable in ontogeny, and not necessarily the normal time of action of a specific gene. In a case of somatic segregation, for example, at whatever stage in ontogeny the segregation occurs, the genes exhibiting mosaicism might act at any time subsequent to the segregation. Only for cases of somatic segregation late in ontogeny is the time of action of the said genes therefore rather precisely determinable. Even so, there is an obstacle to a rigorous conclusion that the time of gene action is here precisely determinable. The recessive allele may in fact have been acting long before the segregation which permits it to escape from its dominant partner and so to manifest itself. Even the dominant allele may have been acting prior to the segregation, if its primary product (after the fashion of kappa in *Paramecium aurelia;* cf. Sonneborn[15]) is unable to persist in the cell in the absence of the gene itself, so that the primary gene product might disappear before the arrival of that time at which it normally would enter the specific morphogenetic system it modifies. The same reasoning holds, *pari passu*, in cases of mosaicism produced by somatic mutation. On the other hand, all such difficulties of interpretation are obviated in the analysis of the action of the suppressor-erupt gene, because at all stages the same genotype is present. It is particularly to be emphasized that the unreduced and metaphase I oöcytes which were irradiated with x-rays were identical in genotype with the fertilized eggs which were treated. The spermatozoa likewise possessed the same genotype, but of course were haploid.

A consideration of this case makes one wonder how many other genes actually initiate action just after fertilization, and to what extent different genes really come into action at different stages throughout the course of development.

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GENE INDUCED MUTATION OF A HERITABLE CYTOPLASMIC FACTOR PRODUCING MALE STERILITY IN MAIZE

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Earlier studies[1-3] have shown that the recessive gene iojap (ij) in maize induces irreversible mutation of plastid primordia. Further work indicates that this gene not only affects plastids but also produces mutation of a second cytoplasmic factor which is concerned with development of the male gametophyte (pollen). When a homozygous iojap plant is used as the egg parent in a cross with a normal green individual the F_1 progeny often consists of white and green-white striped plants in addition to varying proportions of the expected wholly green class. The reciprocal cross invariably yields only normal green offspring. These white and striped plants arise from plastid mutation induced by the iojap gene. The white seedlings come from those egg cells with only mutated plastids, the striped plants from eggs with both normal and mutated plastids, and the green individuals from a cytoplasm with unaffected plastids.

The evidence for the mutation-inducing effect of iojap on a second cytoplasmic factor is as follows. Varying degrees of pollen abortion are found in homozygous iojap plants. If iojap plants are used as the pollen parent in crosses with normal, male-fertile individuals, the F_1 progenies contain only green, wholly male-fertile plants. However, in the reciprocal cross plants which are either completely or partially male-sterile may occur in addition to normal male-fertile individuals. When these male-sterile F_1

plants were pollinated by unrelated male-fertile individuals, the ensuing backcross populations have consisted solely of male-sterile offspring. The failure of the pollen parent to affect the heredity of the male-sterile character indicates that its transmission is cytoplasmic. More meaningful is the fact that the male-sterile phenotype owes its inception to the action of the iojap gene, as is also true for mutation of plastid primordia.

Since the mutation-inducing effect of iojap on plastids resembles that on the cytoplasmic particle concerned with pollen development the question arises whether or not both of these phenotypic characters may be due to the same cytoplasmic factor. It can be stated with some assurance that this is not true. Mutation of the plastid primordia is independent, or largely so, of that of the cytoplasmic factor determining male sterility, for if it were not then only the white plants would, if viable, be male-sterile, the tassel branches on white sectors of striped plants would have aborted pollen while those from green sectors should have normal pollen, and the green siblings should be male-fertile. Such is not the case. Evidence of the particulate nature of this cytoplasmic factor is the occurrence of partially male-sterile plants with diverse percentages of pollen abortion. It is believed that these partially sterile plants arise from egg cells with a cytoplasm containing both normal and mutated particles. Completely male-sterile plants presumably possess only mutated particles while male-fertile individuals have non-mutated particles.

If, as we believe, the cytoplasmic particles determining male sterility are mitochondria, these observations are of some interest in connection with the problem of the differentiation of cytoplasmic particles. Plastids have a genetic continuity; such a fundamental property has been conjectured but never demonstrated for mitochondria. Further, in meristematic cells of maize it is difficult to distinguish between mitochondria and plastid primordia. The statement[4] that plastids arise from mitochondria suggests there is no basic difference between them. It should be emphasized that the postulated role of mutated mitochondria in producing pollen abortion is hardly a demonstrated fact and some other cytoplasmic component may well be involved. However, if our surmise is correct, these investigations provide the first evidence of not only the genetic continuity of mitochondria but also of a fundamental difference between them and plastid primordia. In any event the iojap gene has been shown to induce irreversible mutation of some cytoplasmic constituent other than plastid primordia, and this is in itself of more than passing interest. Needless to say this problem will receive our further attention.

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DIFFERENTIAL REJOINING AS A FACTOR IN APPARENT SENSITIVITY OF CHROMOSOMES TO X-RAY BREAKAGE*

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It has previously been shown[1, 2] that the sensitivity of Trillium chromosomes to x-ray fragmentation varies greatly with the stage of meiosis irradiated. Irradiation of certain stages yields up to fifty times more breakage than that obtained with the same dosage at other stages.[2, 3] The reason for such changes in radiosensitivity of the chromosomes is not known, but it seemed plausible that a comparison of the relative number of certain types of aberrations produced at a highly sensitive stage with those produced at a stage of low sensitivity might yield pertinent information. More specifically, it was decided that aberrations following irradiation at first meiotic metaphase and early post-meiotic interphase would be studied. These are stages of high and low sensitivity, respectively, the former being approximately fifty times as sensitive to breakage as the latter.[2, 3]

Since the work involved in a complete analysis of all aberration types in many hundreds of cells would be prohibitive, scoring was done for only three types; acentric fragments, dicentrics and rings. They were scored at microspore anaphase. The number of fragments served as a rough index of sensitivity and the number of dicentrics and rings as an index of rejoining of broken ends. It was thus possible to compare the amount of rejoining at different stages relative to the sensitivity of that stage. Such a comparison has given information which indicates that rejoining of broken ends probably can and does affect apparent radiosensitivity of chromosomes to x-ray breakage.

Materials and Methods.—Rhizomes of *Trillium erectum* L. obtained from commercial sources were handled as previously described.[4] Test slides were made from one or more anthers just before x-raying to determine the stage. Immediately after treatment the plants were returned to the cold room and left there until they reached the appropriate stage of microspore development. This varied from about five to ten weeks, depending upon the stage and dosage. Permanent propionic or acetocarmine smears mounted in euparal or diaphane were used exclusively. The details of the method used have been published elsewhere.[4]

It should be noted that in *Trillium erectum* L. there is no regular interphase following first division. The first anaphase chromosomes rearrange themselves, and second division follows directly. Post-meiotic interphase is therefore the first interphase to occur after irradiation at first

metaphase. It should also be remembered that in Trillium (and other material) irradiation of metaphase chromosomes does not produce immediate breaks but only potential breaks.[1] True breaks are generally not visible until after the chromosomes have passed through an interphase stage and are approaching metaphase of the next division.

Irradiation was from a Coolidge type tube with Tungsten target operated at a peak of 160 kv. and 10 ma. The half value layer was approximately 0.3 mm. of Cu and its effective wave length was 0.23 Å. Distance from the target was constant in all experiments and time was varied to control dosage. Measurements of dosage were made on an integrating

TABLE 1

SUMMARY OF DATA FROM MATERIAL IRRADIATED AT FIRST MEIOTIC METAPHASE AND SCORED AT MICROSPORE ANAPHASE (ARRANGED IN ORDER OF FRAGMENTS PER 100 CELLS[a])

PLANT AND SLIDE[b]	DOSAGE IN ROENTGENS	NO. OF ANAPHASES ANALYZED	NO. OF ANAPHASE FRAGMENTS		DICENTRICS[c] OBSERVED	DICENTRICS[c] AND RINGS	
			OBSERVED	PER 100[a] CELLS		OBSERVED	PER 100 CELLS[a]
367PO	50	50	53	106.0	1	2	4.0
166FH	25	217	290	133.6	9	14	6.5
166BEDC	25	211	284	134.6	5	10	4.7
144D	50	100	149	149.0	13	15	15.0
143PQ	50	120	179	149.2	20	21	17.5
144N	50	60	96	160.0	9	10	16.7
144E	50	140	227	162.0	29	31	22.1
2375G	100	25	79	316.0	17	21	84.0
917C	200	78	274	351.3	49	76	97.4
2375F	100	25	108	432.0	22	28	112.0
153D	50	32	157	490.6	31	37	115.6
2375P	100	25	143	572.0	42	48	192.0
2150POQ	100	38	246	647.4	43	53	139.5
117E	100	40	263	657.5	33	50	125.0
Total		1161	2548	...	323	416	...

[a] Cells here refer to microspores.
[b] Each number represents a plant, each letter a slide.
[c] Dicentric rings were scored as rings. Tricentrics were scored as 2 dicentrics, etc.

type of dosimeter calibrated against a Victoreen r-meter of known accuracy.

Results.—Data were collected from buds irradiated with 25, 50, 100 and 200 r at meiotic first metaphase (table 1) and with 50, 100, 200, 400, 800 and 1000 r at early post-meiotic interphase (table 2). This range of dosages, plus a certain amount of inherent variability in susceptibility of plants to x-rays, has given a wide range of induced fragment frequencies. The data within each table are arranged in order of ascending fragment frequencies.

Ring-shaped aberrations were scored as rings only when large enough to

TABLE 2

SUMMARY OF DATA FROM MATERIAL IRRADIATED AT EARLY POST-MEIOTIC INTERPHASE AND SCORED AT MICROSPORE ANAPHASE (ARRANGED IN ORDER OF FRAGMENTS PER 100 CELLS)

PLANT AND NO.[a]	DOSAGE IN ROENTGENS	NO. OF ANAPHASES ANALYZED	NO. OF ANAPHASE FRAGMENTS OBSERVED	PER 100 CELLS[b]	DICENTRICS[c] OBSERVED	DICENTRICS[c] AND RINGS OBSERVED	PER 100 CELLS[b]
889E	50	100	9	9	2	2	2
197E	50	100	11	11	2	2	2
513N	50	100	12	12	3	3	3
182FD	50	100	13	13	3	4	4
182NO	50	100	13	13	2	2	2
929O	50	50	7	14	1	1	2
185D	100	223	37	16.6	3	8	3.6
688O	100	111	19	17	2	2	1.8
759R	100	100	18	18	1	2	2
919E	50	100	20	20	1	1	1
467FG	50	100	20	20	4	4	4
513C	50	50	5	10	0	0	0
179C	100	153	31	20.3	3	7	4.6
715CB	100	100	21	21	0	0	0
197G	50	100	21	21	0	0	0
579-O	50	100	24	24	2	2	2
688R	100	89	23	25.8	0	3	3.4
119D	100	82	25	30.5	7	11	13 4
661DEF	100	100	32	32	5	5	5
649-O	100	100	34	34	5	5	5
715N	100	100	35	35	4	4	4
179B	100	42	15	35.7	2	2	4.8
68F	200	100	127	127	31	39	39
378BCDE	400	100	130	130	32	44	44
83ZCZD	200	100	131	131	9	18	18
378BE2	400	100	141	141	31	37	37
88B₁B₂	200	100	145	145	23	26	26
211C	200	200	324	162.0	42	53	26.5
329C-351CD	200	50	137	274.0	37	58	116.0
375D	800	80	246	307.5	72	74	92.5
489C-E	400	58	186	320.7	34	55	94.8
75BCD	200	62	217	350.0	24	36	58.1
371BCFG	1000	90	318	353.3	41	58	64.4
444EFG	200	64	232	362.5	78	110	171.9
377BCD	800	101	412	407.9	96	156	154.5
377M	800	50	220	440.0	50	64	128.0
Total		3455	3411	...	652	898	...

[a] Each number represents a plant, each letter a slide.

[b] Cells here refer to microspores.

[c] Dicentric rings were scored as rings. Tricentrics were scored as 2 dicentrics, etc.

have an unmistakable hole in the center. Questionable cases were not scored. Dicentric rings were counted as rings only and not included

AMOUNT OF REJOINING FOLLOWING IRRADIATION AT METAPHASE I AND INTERPHASE IN TRILLIUM

NUMBER OF FRAGMENTS PER 100 CELLS (MICROSPORES)
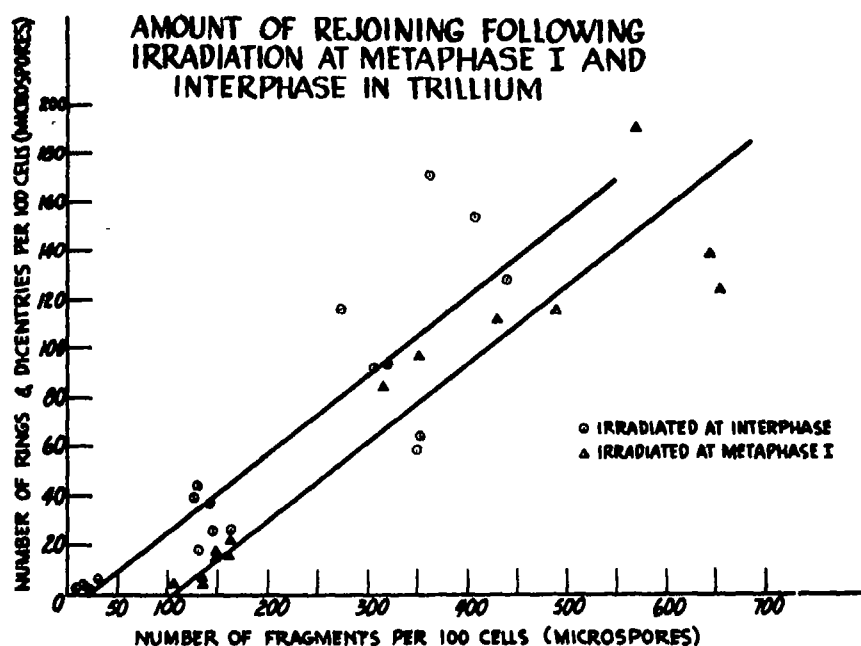
FIGURE 1

with the dicentrics. A few polycentric configurations were observed and these were included with the dicentrics. Tricentrics were counted as two dicentrics, etc.

Using the data given in tables 1 and 2, straight lines were fitted by the method of least squares to the regression of the number of rejoins (dicentrics plus rings) on the number of fragments (Fig. 1). These lines ap-

TABLE 3

SUMMARY OF DATA FROM TABLES 1 AND 2

STAGE IRRADIATED	NO. OF ANAPHASES ANALYZED	NO. OF FRAGMENTS (F)	NO. OF DI-CENTRICS (D)	NO. OF RINGS (R)	$\frac{D}{R}$	$\frac{F}{D}$	$\frac{F}{R}$	$\frac{F}{D+R}$
Metaphase	1161	2548	323	93	3.5	7.9	27.4	6.1
Interphase	3455	3411	652	246	2.7	5.2	13.9	3.8

pear to be very nearly parallel. By means of an analysis of variance the observed deviation from parallelism was found not to be significant. The average regression is significant, and the position or means were also found to differ significantly.

The data from tables 1 and 2 are summarized in table 3. Irradiation at metaphase yielded 3.5 dicentrics for every ring as compared with 2.7

following irradiation at interphase. In the last three columns the ratio of dicentrics and rings to fragments is given for each stage. For each dicentric or ring formed more fragments were counted following metaphase irradiation than after interphase treatment. The combined data (dicentrics plus rings) showed, respectively, 6.1 and 3.8 fragments per rejoin. The data indicate that rejoining, calculated as a function of fragmentation, occurred 1.6 times more frequently after interphase than after metaphase x-ray irradiation. It would, therefore, appear that differences in apparent sensitivity of different stages of cell division are due, in part, to a differential rejoining of broken ends, rather than wholly to a difference in the number of breaks initially induced.

Discussion.—The discussion below will deal mainly with the problem of differential rejoining since the various factors responsible for differences in break frequency have been discussed elsewhere.[1]

The data presented indicate that the number of both dicentrics and rings increases with increasing fragment frequency for both stages irradiated (tables 1 and 2). This is to be expected since the opportunities for rearrangements will increase as the number of breaks per nucleus increases. Actually, therefore, we would expect that the stage of high sensitivity (metaphase) would produce *more* rearrangements per unit number of fragments for a given dosage than a stage of low sensitivity. Since the data show the reverse to be true the observed differences in rejoining are of even greater significance than they may appear at first. This conclusion is in agreement with Bishop's[5] suggestion that peak sensitivity is related to a prevention of restitution but in disagreement with Catcheside's[6] conclusion "that the speeds of reunion and therefore of restitution do not alter much over a great part of the nuclear cycle, and that the internal controlling conditions are relatively constant" (p. 286).

Our results do agree in principle with the results of Bozeman and Metz[7] that the percentage of rearrangements observed in F_1 larvae of irradiated Sciara oöcytes is dependent upon the stage irradiated. They found different types of rearrangements to reach maxima at different stages of division. Adjacent repeats were highest at prophase and inversions were highest at anaphase. Deletions were high at prophase and metaphase and much lower at anaphase.

Changes in the definitive number of aberrations observed following a given dose of radiation at different stages may be due to (1) a difference in the initial number of breaks (or potential breaks) induced or to (2) a difference in the amount of rejoining of open breaks or (3) to a combination of both. The data presented show that following irradiation of different stages a difference in the amount of rejoining does occur but that it is not sufficient to account for the observed change in sensitivity based on fragment counts. It may, therefore, be concluded that a difference in the

number of initially produced breaks (or potential breaks) must also be concerned in determining the ultimate yield of breaks.

Various estimates have been made of the percentage of initially produced breaks which rejoin in the original sequence.[8–11] The present data indicate that the amount and kind of detectable rejoining varies with the stage of division irradiated and suggests that undetectable rejoins probably also vary with stage. Since other factors (temperature,[13–14] infra-red[15, 16] and ultra-violet[16, 17]) are also known to have an effect on rejoining, it would appear that rejoining is a highly variable process. To further complicate the situation rejoining is considered to be non-random in Tradescantia.[19] It would thus appear that the relationship between initial breakage and the amount and kind of rejoining is highly variable. It follows that sizeable errors might be introduced by carrying over data on a particular type of aberration obtained from one organism (or stage of division) to another. Unfortunately this is sometimes done.

The inverse relationship between breakage sensitivity and tendency of broken ends to rejoin may be of significance in the study of changes in the frequency of certain types of aberrations. For instance, if the capacity for rejoining is automatically reduced when many breaks occur in a single nucleus, a high break frequency would not produce the expected percentage of complex aberrations. This would account for the observed decrease in 2-hit aberrations at higher dosages,[20] as well as reduced percentage of dicentrics and rings observed (see above) at the most sensitive stages in Trillium.

In the presence of a low frequency of rejoining we would expect an increase in the percentage of acentric fragments or deletions and hence greater lethality. Therefore, aberrations recovered in the offspring of irradiated organisms would not necessarily represent a reliable picture of total chromosome breakage since cells irradiated in the most sensitive stages would be the least likely to produce viable gametes or progeny. It seems likely, therefore, that a sensitivity curve based solely on recoverable rings, translocations, inversions, etc., would differ from a curve based on the terminal and interstitial deletions (cf. 7, 21). Actually a curve based on the latter would probably be a more reliable indication of expected mortality since aberrations involving rejoins would be, as a whole, much less lethal than the loss of a portion of a chromosome.

Since mutations have been presumed by a number of investigators to be associated with primary breaks, it would be interesting to know whether gene mutation rate also varies widely with the stage of division irradiated. So far this aspect of mutation has not been sufficiently well investigated. It has been shown that x-ray induced mutation rate is higher at first metaphase than at early prophase in Prunus and Oenothera[22] and that it varies considerably at different stages of microsporogenesis in Tradescantia.[24, 25]

It is also higher in young than in mature pollen grains of *Antirrhinum majus*.[24] It thus seems plausible that mutation rate will be found generally to vary with the stage irradiated. It should therefore be realized that estimates of gene size based on irradiation of a particular stage (e.g., mature sperm of Drosophila) do not necessarily represent the true size of a "working" gene. If the changes in mutation sensitivity are as great as those of break sensitivity, estimates of gene size may well be off by a factor of several hundred or even several thousand per cent. In this connection, it is of interest to note that sensitivity volume of a virus actually does change during various stages of growth.[26]

In the present study the two stages investigated are known to differ with respect to the following factors: matrix, nuclear membrane, proximity of different chromosomes or chromosome parts, spiralization and stress of spiralization, degree of polarization, amount of attraction or repulsion, cytochemical constitution and physiological activities. The relative importance of these various factors in determining the behavior of chro o-somes following irradiation must await further investigation.

Summary.—Differential rejoining has been studied in microspores of *Trillium erectum* following x-irradiation at stages of high and low apparent sensitivity to breakage. The stages irradiated were meiotic first metaphase and early post-meiotic interphase. Fragments, dicentrics and rings were scored at microspore anaphase. The numbers of dicentrics and rings observed were used as a measure of rejoining. The data indicate a significantly higher amount of rejoining in cells irradiated at early interphase than in those irradiated at meiotic metaphase. However, the increase in rejoining is not sufficient to account for the observed decrease in fragmentation.

It is therefore concluded that the observed difference in sensitivity of the two stages studied is due in part to a difference in number of primary breaks (or potential breaks) and in part to a difference in the amount of rejoining which subsequently occurs. In the material studied a high sensitivity to primary breakage is associated with a low frequency of rejoining and vice versa. Certain implications of these conclusions on calculations of mutation rate and gene size are discussed.

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THE CONSTANCY OF DESOXYRIBOSE NUCLEIC ACID IN PLANT NUCLEI*

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For a number of years considerable interest has been centered in the role of nucleic acids in cellular processes.   Recently desoxyribose nucleic acid (DNA) has been shown to possess interesting characteristics that have led several workers to consider it an essential component of the gene.[1-3] DNA is probably a universal constituent of plant and animal nuclei.   Its low turnover rate to radioactive phosphorus and nitrogen, in non-dividing tissues, is evidence for a chemical stability considerably greater than that of other cell components.[4,5]   Moreover, recent analytic data on the actual amounts of DNA within nuclei have suggested that it possesses a quantitative stability as well.

Computations on the amount of DNA per nucleus have been made in two ways.   Chemical analyses of large numbers of cells, with the number present estimated by sample counts, have given the average amount of

DNA, per nucleus, for many thousand cells. Light absorption measurements made through a microscope have, on the other hand, yielded data on individual nuclei. Both methods involve technical difficulties and are open to certain criticisms. Nevertheless both biochemical[1, 3, 6] and microscopic[4, 7, 9] measurements by a number of different investigators have supported the concept first proposed by Boivin, Vendrely and Vendrely[1] that within the tissues of an organism the actual amount of DNA per nucleus is apparently constant.

Boivin, *et al.*, have considered that all somatic cells of an animal possess the same amount of DNA, with the gametes containing half this value. A number of discrepancies from this simple relation have been reported. Although some of these may be attributable to errors in the analytical techniques employed, at least two processes obviously involve naturally occurring variations. Ris and Mirsky[7] first showed that DNA classes with the ratio 1:2:4 occur in the rat liver associated with polyploid nuclei. It was later found[3] that such DNA classes, apparently associated with polyploidy and polyteny, occur in a variety of animal tissues. Variation of another type occurs in association with the mitotic cycle. Since DNA in several animal tissues has been found to increase in interphase preceding cell division to twice the diploid amount, interphase nuclei of dividing tissues may contain anywhere from two to four times the amount found in the haploid sperm.[3] These variations in the amounts of DNA, associated with mitosis or DNA classes, cannot be considered in disagreement with the basic tenets of Boivin's theory. It is clear that, at least in many different animal tissues, the amount of DNA per nucleus is under rather definite quantitative restrictions. Interphase nuclei of non-dividing tissues, with a few possible exceptions, have been found to contain an amount of DNA approximately 2, 4, 8, etc., times that found in the gametes. A haploid amount of DNA has been found in sperm,[1, 2] spermatids[2, 9, 10] and male and female pronuclei.[8] In general these estimates of the nucleic acids in cells are at present accurate to 10 or 20%. The question of how precisely the quantitative relations are obeyed must await more accurate techniques.

The quantitative behavior of DNA in plant tissues has as yet received little attention. Schrader and Leuchtenberger[11] have recently shown that the amount of DNA varies from tissue to tissue in Tradescantia. The data presented, although they clearly show that the amount of DNA is different in different tissues, are not extensive enough to demonstrate the type of variability. Does DNA tend to occur in the well-marked constant units found for a number of animal tissues, or, as might be inferred from the work of Schrader and Leuchtenberger, does it fail to follow any definite quantitative pattern? The present work was primarily designed to answer this question.

Material and Methods.—Two plant species have been studied in detail and two others briefly. Plants of *Tradescantia paludosa* were kindly provided by Dr. J. M. Beal of the University of Chicago. Corn plants (*Zea mays*) were obtained from several sources, particularly commercial Golden Bantam, and three strains from the collection of Dr. M. M. Rhoades of the University of Illinois. Plants of *T. canaliculata* were collected in the Chicago region, and cultivated plants of *T. "virginiana,"* differing slightly from the typical native form, came from a Chicago garden. All material was fixed in neutral 50% formalin (one part of the stock 40% formaldehyde solution to one part of distilled water, with calcium carbonate added) immediately after removal from the plant. Pieces were small, with the smallest dimension rarely exceeding 1 mm., and buds were opened to permit rapid pentration. Fixation was for at least three hours. Material was thoroughly washed, sectioned in paraffin, and stained with the Feulgen reagent for one hour after an hydrolysis in normal hydrochloric acid of 14 minutes at 60°C. In the present study, wherever possible, all tissues to be compared were mounted together on the same slide, and where this was not done, a section of tissue previously studied was mounted beside the unknown. Control sections in five of the eight slide series studied gave essentially similar values. In the remaining three the Feulgen intensity of the control sections were slightly below that usually obtained, and all values from these series were consequently raised.

The amount of Feulgen dye in individual nuclei was estimated by photometric determinations made through a microscope. The technique was approximately as described previously[2, 12] with the following exceptions: Essentially monochromatic light was isolated by a Beckman spectrophotometer with a slit width of 0.1 or 0.03 mm. Measurements were made with a Leitz achromatic-aplanatic condenser, N.A. 1.4, a 90 × Leitz 2-mm. oil immersion apochromatic objective, N.A. 1.32, and a 20 × Bausch and Lomb coated hyperplane ocular, containing an iris diaphragm to minimize distortion from internal reflection. The microscope image, enlarged 1000 times, was projected on a field diaphragm, which allowed an area 2, 3, 4 or 5 mm. in diameter, taken in the center of the nuclear image to fall on the phototube. Measurements were made with a battery-powered 1P21 electron multiplier phototube, with output leads connected to a Farrand type B control unit and a Rubicon galvanometer. Measurements of corn tissues were made at the absorption peak of the Feulgen dye, 560 mμ. Tradescantia nuclei at this wave-length were too dark to measure accurately, and consequently were measured considerably off the maximum absorption, at 615 mμ. The extinction at this wave-length was about 22% of that at 560 mμ.

Photometric determinations of biological material are subject to a variety of technical variables.[3] The most important of these in the present work were probably caused by the irregular distribution of the stainable com-

ponents in the nucleus and inaccuracies in estimating nuclear volume. A strong formalin fixative, where rapid penetration is aided by using small pieces of tissue, tends to keep the chromatin in its natural extended state. Where the nuclei measured were markedly irregular, e.g., in meiotic diakinesis, measured values tended to be low. The apparatus was calibrated
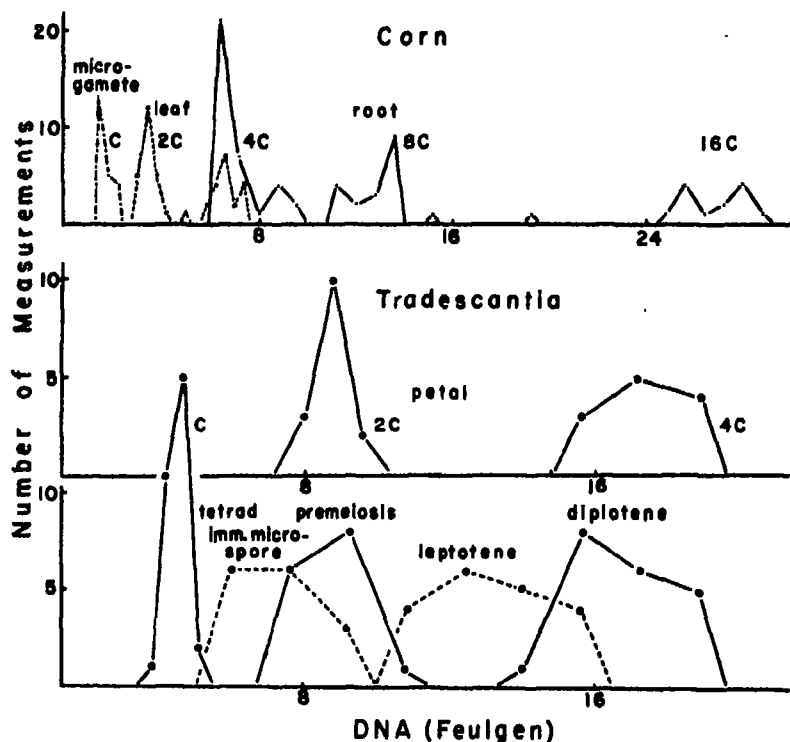
DNA (Feulgen)

FIGURE 1

Distribution of DNA (Feulgen) measurements on individual nuclei of corn and Tradescantia tissues. The amount of DNA is shown in arbitrary units. *Upper graph:* Corn microgamete nuclei from pollen grains (dashed lines), leaf nuclei (dotted lines) and nuclei from corn root zone of elongation (solid lines). *Middle graph:* Tradescantia petal nuclei from mature flower. *Lower graph:* Stages in Tradescantia pollen formation from developing anthers, showing measurements on premeiosis (preleptotene), leptotene, diplotene, tetrad and immature microspore stages.

as previously, and the non-specific light loss was found to be negligible by measuring unhydrolyzed controls.

The advisability of using the Feulgen reaction for quantitative microphotometric determinations of DNA has been discussed by a number of workers. It is now generally agreed that, where staining procedures are properly carried out, the Feulgen reaction can give an accurate relative

estimate of the DNA in nuclei.[4, 7, 10]   Since the actual intensity of the dye produced can be markedly altered by such factors as the type of fixative used, size of the tissue fixed and slight changes in hydrolysis conditions no attempt has been made here to convert the data presented into absolute amounts of DNA.   All values are given in the arbitrary units used elsewhere.[4, 8]   The measured extinction (E) of a central region 2 to 5 microns in diameter of an uncut nucleus has been multiplied by the squared radius of the measured area (C^2) and divided by the fraction (F) of the total nuclear volume included in the measured region.   Units $= \dfrac{EC^2}{F}$, where $F =$

$\dfrac{R^3 - (R^2 - C^2)^{3/2}}{R^3}$, and R is the radius of the nucleus.   Markedly aspherical nuclei were not measured.   Where nuclei were slightly ellipsoid, R was taken as the mean of major and minor axes.

Results.—(A) Non-Dividing Tissues: Photometric measurements made on tissues where mitoses were uncommon tended to fall in certain well-marked classes.   Means of these classes fit in the series $1:2:4:8:16:32$. The distributions of measured values from corn leaf and root, and Tradescantia petal are shown in figure 1.   Means for all measurements are given in tables 1 and 2.   The values are expressed as the total number of dye molecules per nucleus, in arbitrary units, and thus constitute a relative estimate of the DNA in nuclei.   In similar tissues the arbitrary units are about 2.5 times higher for Tradescantia than for corn.   Since the Tradescantia tissues were measured at a wave-length giving only about 22% of maximum extinction, these nuclei contain approximately 10 times the DNA found in corn.

The lowest values for Tradescantia have been found in the young microspore nuclei (tetrad stage), young generative nuclei and tube nuclei; and for corn in the microgamete nuclei of mature pollen, all presumably haploid.   Most nuclei in both species were found to have twice ($2C$) or four times ($4C$) the haploid amount.   Nuclei falling in class $8C$ have been found in Tradescantia stamen hairs, corn root and root cap and in the scutellum nuclei of the corn kernel.   In the root and scutellum $16C$ nuclei also occur.   In a few tissues, i.e., the root cap and zone of elongation in corn, and in the mature stamen hairs in Tradescantia, class $2C$ nuclei are rare and almost all nuclei belong to the higher classes.   In young stamen hairs, however, class $2C$ cells are common.   Measurements on the aleurone and endosperm of the corn kernel, tissues long known to be triploid through the joining of one microgamete with the $2N$ endosperm nucleus, fell in the series $3:6:12:24$.   Most aleurone nuclei in the kernels studied fell in class $6C$.   Endosperm nuclei were measured in young ears, since those of the mature kernel are highly irregular.   The smaller classes tended to be peripheral.

In the corn root zone of elongation most nuclei fell in classes 4C and 8C. However, in certain rows of cells forming the major vessels of the root, larger classes (up to 32C) were found.   When all the nuclei in such a vessel were measured in order from the root tip back to about 1500 μ from the tip,

TABLE 1

AVERAGE AMOUNTS OF DNA (FEULGEN) PER NUCLEUS IN VARIOUS TISSUES OF CORN (Zea mays)

CELL TYPE	DNA CLASS	DNA IN ARBITRARY UNITS	STANDARD ERROR	NO. MEASURED
Microgamete nucleus (interphase)	C	1.6*	0.06	22
Leaf				
(interphase)	2C	3.4*	0.05	23
	2C → 4C	4.8*	..	1
	4C	6.6*	0.12	19
(prophase)	4C	6.9	0.12	10
(telophase)	2C	3.2	0.09	12
Root cap (interphase)	4C	6.9	0.12	15
	8C	12.6	0.33	22
Root elongation zone (interphase)	4C	6.6*	0.06	29
	4C → 8C	8.7*	..	6
	8C	12.5*	0.27	19
	8C → 16C	20.2*	..	2
	16C	26.1*	0.45	17
	16C → 32C	33.8	..	2
	32C	49.0	..	6
Root meristem				
(interphase)	2C → 4C	5.4*	0.21	36
(prophase)	4C	6.4*	0.09	15
(telophase)	2C	3.2*	0.05	20
Embryo (interphase)	2C	3.6	0.07	15
	4C	7.1	0.13	15
Scutellum (interphase)	2C	3.3	0.09	20
	4C	6.4	0.11	40
	8C	12.6	0.24	15
	16C	26.2	..	5
Aleurone (interphase)	3C	4.8	0.07	15
	6C	10.1	0.20	40
	12C	20.5	0.60	15
Endosperm (interphase)	3C	5.0	0.06	15
	6C	9.3	0.14	17
	12C	19.1	0.60	16
	24C	38.0	1.20	10

* Data graphed in figure 1 or figure 2.

all classes from 4C to 16 or 32C were usually represented, in ascending order. From 6 to 12 nuclei have been found together from each class, and between these groups anywhere from 0 to 6 intermediate values have been obtained. It is thus likely that these large vessel cells, while remaining in interphase undergo a periodic DNA doubling.   Values from a few such series are com-

bined in figure 1 and table 1; in table 1 only values falling outside the expected interclass variability have been considered as intermediate.   More work on this process is in progress.

Only one intermediate value has been obtained from leaf tissue in more than 150 measurements, and this came from the leaf base where a few mitoses were present.   In the differentiated leaf, where cell division is absent, no intermediates have been found.   It seems probable that intermediate amounts occur only when cells are synthesizing DNA for cell division (see below) or periodic DNA doubling.

(*B*)   *Dividing Tissues:* The quantitative changes in DNA during the mitotic cycle were studied in corn root and leaf meristem, and in the root
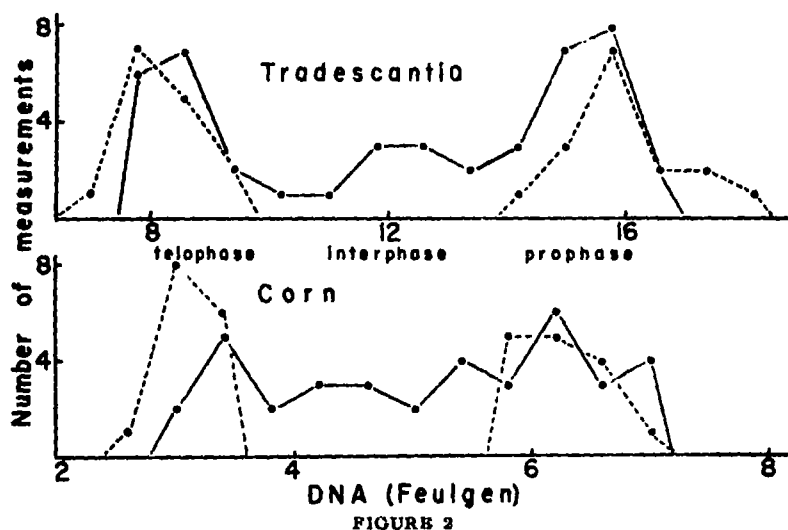
FIGURE 2

Distribution of DNA (Feulgen) measurements on individual nuclei from the root meristem of corn and Tradescantia.   The amount of DNA is shown in arbitrary units.   Interphase nuclei (solid lines), prophase nuclei (dotted lines at right), and telophase nuclei (dotted lines at left).

meristem and sporogenous tissue of Tradescantia.   In all these tissues the process was essentially the same, paralleling that described previously for animals.[8]   Where mitotic figures were common, measurements on interphase nuclei scattered widely between classes 2C and 4C (Fig. 2).   Prophase values fell in 4C and telophases in 2C.   Late prophase, metaphase and anaphase cells were too irregular to measure, but the behavior of DNA in these stages can easily be inferred.   Apparently DNA increases during interphase to double the common diploid amount, reaching the 4C value at or before the visible beginning of prophase.   During prophase and metaphase probably no DNA is synthesized.   The 4C amount is then cut in

two at anaphase, and the telophase nuclei each possess the $2C$ value. In the tissues studied no $8C$ or $16C$ prophase stages have been found, although

TABLE 2

AVERAGE AMOUNTS OF DNA (FEULGEN) PER NUCLEUS IN VARIOUS TISSUES OF *Tradescantia paludosa*

CELL TYPE	DNA CLASS	DNA IN ARBITRARY UNITS	STANDARD ERROR	NO. MEASURED
Leaf (interphase)	$2C$	8.5	0.07	20
Root meristem				
(interphase)	$2C \rightarrow 4C$	13 1[a]	0.47	30
(prophase)	$4C$	16.2[a]	0 29	15
(telophase)	$2C$	8.3[a]	0.16	15
Tapetum (interphase)	$2C$	8 5	0.10	33
	$4C$	16.4	0 36	14
Petal (interphase)	$2C$	8.6[a]	0.13	15
	$4C$	16.9[a]	0 27	15
Stamen hairs (interphase)	$2C$	8.5	0.26	10
	$4C$	16.6	0.26	21
	$8C$	33 6	0.65	15
Sporogenous tissue				
(interphase)	$2C \rightarrow 4C$	13 1	1.00	15
(prophase)	$4C$	16.0	0.24	15
(telophase)	$2C$	8.1	0.19	15
Microspore mother cells				
(preleptotene)	$2C$	8.7[a]	0.20	15
(leptotene)	$2C \rightarrow 4C$	12.6[a]	0 39	15
(leptotene)	$2C \rightarrow 4C$	13.0	0.54	20
(zygotene)	$4C$	16.1	0.20	20
(pachytene)	$4C$	16 8	0.39	15
(diplotene)	$4C$	16.9[a]	0.28	20
(diakinesis)	$4C$	16.3	0.25	20
Microspores				
(tetrad stage)	C	4.4[a]	0.04	30
(early interphase)	C	4 0	0 10	20
(late interphase)	$C \rightarrow 2C$	5.9	0.34	10
(late interphase)	$C \rightarrow 2C$	7.4[a]	0.24	25
(prophase)	$2C$	9.2	0 20	15
Pollen tube nuclei				
(early interphase)	C	4 2	0.09	15
(late interphase)	C	4.1	0.07	27
Generative nuclei				
(early interphase)	C	4.3	0.11	14
(late interphase)	$2C$	8.5	0.08	16

[a] Data graphed in figure 1 or figure 2.

their occurrence might be expected in connection with the division of $4C$ or $8C$ nuclei. In the corn root zone of elongation where most interphase nuclei fall in class $4C$, cells apparently proceed directly into a $4C$ prophase without DNA synthesis, the $4C$ level then being restored in the following

interphase. In the stamen hairs of Tradescantia most cell division was found to take place in developing buds, where $2C$ nuclei were common, following the usual pattern. No division stages were seen in the $4C$ or $8C$ nuclei from mature flowers.

(C) *Meiosis:* The behavior of DNA during pollen formation was studied only in Tradescantia, since the chromosomal material during most meiotic stages of corn was found too irregular for measurement. Anthers of Tradescantia, when first differentiated, are filled with rapidly dividing sporogenous cells. Measurements of these cells, in young anthers, tended to follow the pattern found in other dividing tissues (table 2). Prophases fell in $4C$, telophases in $2C$, and interphases were scattered in between. The beginning of meiosis is marked by general cessation of mitotic activity in the anther, except at the periphery. The nuclei measured in this pre-leptotene resting stage, before the thread-like chromosome structure becomes apparent, fell in class $2C$. During the subsequent leptotene, microspore mother cells were found to increase in amount of DNA so that measurements on this stage were intermediate between $2C$ and $4C$ (Fig. 1). The next stage measured was late zygotene, where only a few unpaired strands were visible. In these cells the DNA had approximately doubled $(4C)$, and throughout the rest of the meiotic prophase no further increase was found. Immediately after the second maturation division, while still in the tetrad stage, the microspore nuclei fell in class C. Measurements on microspores from three later stages of development indicate that a comparatively long period ensues during which the microspore contains the class C amount, followed by a fairly rapid increase prior to mitosis. Data for microspores from one anther, intermediate between C and $2C$, are graphed in figure 1. At early prophase of the microspore division nuclei fall in class $2C$ and are divided into tube and generative nuclei, each with the class C amount. Some time before anthesis the generative nucleus increases to $2C$, but the tube nucleus remains at the C amount. In the mature pollen most generative nuclei become very elongate and are thus impossible to measure, but a few continue to be spheroidal, and in these the DNA can be determined. The two microgamete nuclei resulting from division of the generative nucleus were not measured in Tradescantia, but the microgamete nuclei were studied in corn and fell in class C (Fig. 1 and table 1).

The course of DNA in Tradescantia meiosis can thus be outlined briefly as follows: The earliest microspore mother cells fall in class $2C$, increasing during leptotene and possibly also during zygotene to $4C$ for the remaining stages of the meiotic prophase. The four tetrad nuclei resulting from the maturation divisions each have the C amount, increasing to $2C$ before the microspore mitosis. This division results in class C tube and generative nuclei, the latter increasing to $2C$ before anthesis. The generative nucleus apparently divides to form two haploid (C) microgamete nuclei.

(D) *Strain and Species Differences:* As mentioned above, nuclei of *Tradescantia paludosa* contain about ten times the DNA found in corn nuclei of the same class. The amount of DNA in *T. paludosa* leaf nuclei was compared with that found in two other closely related Tradescantia species (table 3). Both species have a haploid chromosome number of 12, twice that of *T. paludosa*, and thus it is not surprising to find the diploid nuclei contain about twice the DNA. Interspecific variation in amounts of DNA has been reported for animals and is not unexpected in view of the deletions, duplications, polysomaty, etc., considered to accompany evolution. On the same basis one would also expect the amount of DNA per nucleus might differ to some extent in various strains of the same species. To test this possibility similar tissues from two corn strains, differing in amount of heterochromatin, were mounted together and measured. Strain *A* (table 3) contained several *B* chromosomes and knobs, and the interphase nuclei showed the chromocenters associated with them.[13] Strain *B* had no *B* chromosomes and contained only a small amount of

TABLE 3

AVERAGE AMOUNTS OF DNA (FEULGEN) PER NUCLEUS IN TISSUES OF TWO STRAINS OF CORN AND THREE SPECIES OF TRADESCANTIA

	LEAF			ROOT		
	DNA IN ARBITRARY UNITS	STANDARD ERROR	NO. MEASURED	DNA IN ARBITRARY UNITS	STANDARD ERROR	NO. MEASURED
Corn strain A	6.4	0.12	15	6.6	0.08	15
Corn strain B	7.2	0.10	15	7.4	0.12	15
T. paludosa	8.5	0.08	25			
T. "virginiana"	16.8	0.20	25			
T. canaliculata	16.1	0.18	25			

heterochromatin on chromosome 6. Class 4C nuclei, from root and leaf, showed a difference in DNA between strains of about 10%.

Discussion.—The data presented indicate that DNA follows quantitative restrictions of the same general type reported for animal tissues. Three points may be stressed: (1) The amount per nucleus shows a marked step-like occurrence. (2) There is a duplication with mitosis and a reduction with meiosis. (3) Since species and strains have characteristic amounts of DNA it is apparent that the quantities involved are directly associated with the genotype. At least for the present these factors seem best interpreted by considering DNA as a component of the gene.

A considerable amount of recent cytological evidence has accumulated that points to the occurrence of "supernumerary chromonemal reproductions" as they have been called by Lorz[14] in numerous plant tissues. In several instances chromosomes have been described as 2, 4 or 8 stranded (polytene).[15,16] Endomitotic cycles, such as those described in the tapetum of Spinacia[17] or tomato,[18] are known to cause doubling of the chromo-

some number, and where differentiated resting nuclei have been stimulated to divide by auxins[19] or other treatment, polyploid nuclei with 2, 4, 8, etc., times the diploid chromosome number have been found. As pointed out by Schrader and Leuchtenberger it seems likely that the occurrence of varying amounts of DNA is associated with such factors.

The nuclei measured in the course of the present work naturally represent an extremely small sample. Nevertheless.it. is interesting that so few intermediate values have been found in non-dividing tissues. This would seem to indicate that the "endomitotic" processes, during which DNA doubling occurs, are comparatively rapid, and that unsynchronized chromonemal reproductions of the type seen in Rhoeo[19] are rare in the tissues studied. They may be more common in the older nuclei, which are often too irregular to measure.

The conclusion seems unavoidable, from both cytological and photometric evidence, that many, and in some tissues most, cells typically contain multiple chromonemal sets. The role played by these cells in the economy of the organism can at present only be conjectured. By analogy with the situation in autopolyploid plants one might expect the physiological balance to be altered. It has often been suggested that endomitotic gene doubling is associated with differentiation.[20, 21] However, in Tradescantia stamen hairs, as well as in some mammalian tissues, the higher classes do not appear until differentiation is completed.

Summary.—Photometric determinations on individual Feulgen-stained corn and Tradescantia nuclei support the view that DNA occurs in well-marked units characteristic of the strain or species. Nuclei with 2, 4, 8, 16 or 32 times the haploid (microgamete) value occur. Preceding mitosis DNA increases in interphase to twice the dipoloid amount. In meiosis the DNA is reduced, so that the microgamete contains half the diploid value.

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ALLELISM OF SECOND CHROMOSOME LETHALS IN D. MELANOGASTER*

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Our knowledge of the genetic composition of populations, other than that of human blood group genes, is based primarily on lethal chromosomes from populations of Drosophila. Paradoxically, because of the advanced genetic techniques in Drosophila, our information for species of this genus consists of *chromosomal* frequencies although the dynamics of population genetics depends upon *gene* frequencies. It would be possible to analyze Drosophila populations for specific gene loci but it has proved more profitable to evaluate the easily collected lethal chromosome data by estimating the number of loci on a given chromosome at which lethal alleles may exist. This estimation, which can be made by determining the frequency of allelism between lethals of independent origin, has been made by Wright[1,2] for the third chromosome of *D. pseudoobscura* (285-289 loci) and by Ives[3] for the second chromosome of *D. melanogaster* (495 loci). In connection with experimental populations which are exposed to continuous gamma irradiation, it has been necessary to determine the number of loci which are capable of mutating to lethality under the influence of these radiations.

Flies of the Oregon-R strain of *D. melanogaster* carrying lethal-free second chromosomes were placed in a population cage and were allowed to oviposit on food in small plastic cups throughout the day (8 hrs.) or overnight (16 hrs.). (See Wallace[4] for a detailed description of the cages and the cups.) At the end of each egg-collecting period, a fresh cup was exposed to the parental flies, and the old cup, with its eggs, was placed in a cage which encircled a 500-mg. radium bomb. To keep the developing flies of each cup separate from the rest, a thin-walled plastic tube was inserted into the food of each cup and was plugged at its free end with cotton.

The flies developing under these conditions were exposed throughout their developmental stages and for part of their adult life to a constant dose of gamma rays of approximately 5 r per hour. After 18 days (426 hrs. or 2175 r, average), males were removed and mated individually in vials with CyL/Pm females. (The CyL chromosome carries the dominant genes Curley and Lobe and two inversions which suppress most of the crossing-over.) Several CyL/+ F1 males of each culture were mated singly in vials with CyL/Pm females, and the CyL/+ F2 males and females of each culture were inbred in vials to determine the lethal mutation rate. If a lethal had been induced on a treated chromosome, this was noticed by the absence of wild-type flies in the F3. Each suspected lethal was confirmed by mating for an additional generation in a regular culture bottle. After confirmation, 125 lethals which had arisen in different original males and, therefore, were independent in origin, were subcultured in four bottles in order to obtain flies for the allelism tests. With the exception of the latter tests which were kept at room temperature ($22° \pm$) all phases of the experiment were carried out at 25°C.

To determine the frequency of induced mutations, 3772 second chromosomes derived from 336 treated males were analyzed; 456 were lethal. The frequency of lethal chromosomes, then was $12.09 \pm 0.53\%$. The frequency of lethal genes, calculated by means of the Poisson distribution, was $12.89 \pm 0.60\%$.

The tests for the frequency of allelism were made by intercrossing CyL/+ flies from 100 lethal cultures of independent origin. (The remainder of the 125 cultures originally chosen either produced too few flies for the required matings or gave rise to small numbers of wild-type flies which could have obscured the test.) Fourteen of the 4950 $\left(\dfrac{100 \times 99}{2} \right)$ matings failed to produce wild-type flies. The probability, then, that one tested chromosome was allelic to another is 0.28%. (The limits of the 95% confidence interval of this frequency are 0.16% and 0.48%).

The minimum number of loci, n, capable of mutating to lethality is the inverse of the probability that one lethal gene is allelic to another. This number can be calculated from the frequency of lethal chromosomes ($a = 12.09 \pm 0.53\%$), the frequency of lethal genes ($b = 12.89 \pm 0.60\%$) and the frequency of allelism between lethal chromosomes ($c = 0.28\%$ with limits 0.16% and 0.48%): $n = b^2/ca^2$. The most probable minimum number calculated from our data is 400. By substituting the most divergent values of a, b and c into the equation, the limits of the minimum number may be estimated as 234 and 718.

The distribution of allelic lethals among the 100 chromosomes which were tested gives a quasi-independent confirmation of the above estimate of the minimum number of loci. In one case chromosome A was allelic to both

chromosomes B and C, but B and C were non-allelic, therefore, it is known that the tests for allelism involved lethal genes at 101 loci. Among these 101 lethals there were 73 which occurred only once and 14 which occurred twice. For any given number of potentially lethal loci it is possible to predict the distribution of singles, pairs and triplets among 101 independently chosen lethals by means of the Poisson series. If $n = 234$, there should be 65.6 singles, 14.2 pairs and 2.0 triplets; if $n = 400$, these values should be 78.4, 9.9 and 0.8; if $n = 718$, 87.7, 6.2 and 0.3. The expected distribution when $n = 718$ is significantly different from the observed distribution; it is probably that the minimum number of lethal loci lies nearer the calculated 400.

It is of interest to note the similarity between the number of loci capable of mutating to lethality under the influence of gamma rays (400 with limits of 234 and 718) and the number capable of mutating spontaneously (495 with limits of 285 and 705). This agreement indicates that these loci are so situated that an ionizing particle is likely to affect only one at a time. Since nearly 2000 bands have been recorded in the second chromosome of *D. melanogaster* in salivary preparations and since induced lethals have frequently proved to be small deficiencies (Slizynski[6]), it seems likely that loci mutating frequently to lethality may be separated from one another by material relatively inert in this respect.

In conclusion, several points should be emphasized. The 400 loci calculated above represent a minimum number of loci which can mutate to lethality. The maximum number could be substantially larger but, nevertheless, remain undetected because of great differences in the mutation rates of different loci. It should be noted, too, that observed allelism does not prove identity; overlapping deficiencies may act as allelic lethals. These considerations have been pointed out by Wright[1] in his analysis of Dobzhansky's data.

Summary.—Through an analysis of the frequency of allelism of 100 second chromosome lethals induced in *D. melanogaster* by chronic gamma-ray treatment, it has been estimated that the minimum number of loci capable of mutating to lethality under these conditions is 400 (234–718).

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COHOMOLOGY THEORY OF ABELIAN GROUPS AND HOMOTOPY THEORY II

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1. Generic Homology Groups.—The cohomology groups of a group II are the groups of the cell complex $K(\text{II}, 1)$ which has as its cells in each dimension $q \geqq 1$ the q-tuples $[x_1, \ldots, x_q]$ of elements $x_i \, \epsilon \, \text{II}$, with $\partial[x] = 0$ and

$$\partial[x_1, \ldots, x_q] = [x_2, \ldots, x_q] + \sum_{i=1}^{q-1} (-1)^i [x_1, \ldots, x_i x_{i+1}, \ldots, x_q] + (-1)^q [x_1, \ldots, x_{q-1}]. \quad (1)$$

This boundary formula does not use inverses of the letters x_i, and no letter x_i is repeated in any one cell of the boundary. These two properties may be conveniently expressed in the complex $K(F, 1)$ constructed from the free group F with a denumerable set of free generators $g_1, g_2, \ldots$. Call an element of F *generic* if it is a product $x = 1g_{i_1}g_{i_2}\ldots g_{i_k}$ of $k \geqq 0$ distinct generators, and call two generic elements x and y *disjoint* if they involve no generator in common. A cell $[x_1, \ldots, x_q]$ is generic if the entries $x_1, \ldots, x_q$ are generic and pairwise disjoint. Then formula (1) shows that the boundary of any generic cell is a linear combination (with integral coefficients) of generic cells. Consequently the generic cells span a subcomplex $K(F^*, 1)$ of $K(F, 1)$.

The usual proof[1] that the cohomology groups of a free group F are zero in all dimensions greater than 1 gives the integral homology groups of this "generic" complex $K(F^*, 1)$ as

$$H_q(K(F^*, 1)) = 0, \qquad q > 1 \qquad (2)$$

$$H_1(K(F^*, 1)) = F_a, \qquad (3)$$

where F_a is the free abelian group on a denumerable set of generators g_i',

$g_3, \ldots$, and the isomorphism in (3) is given by the mapping $[x] \to \phi x$, where $\phi : F \to F_e$ maps each g_i into g_i.

The geometric applications (in particular Theorem 4 below) indicate that the cohomology groups appropriate to an abelian group Π will be those of a suitable cell complex which has the formal properties (2) and (3) relative to the generic elements of the free *abelian* group F_e. The first stage in the construction of such a complex $A(\Pi)$ will be the complex $A^0(\Pi) = K(\Pi, 1)$. The generic subcomplex $A^0(F_e^*)$ satisfies (3) but not (2). Indeed, if g and h are any two generators of F_e, then, because of the commutative law, $\partial[g, h] = [h] - [gh] + [g] = \partial[h, g]$. Thus

$$[g, h] - [h, g] \tag{4}$$

is a 2-dimensional generic cycle; it is not a boundary. Similarly, for any three distinct generators g, h and k,

$$[g, h, k] - [g, k, h] - [h, g, k] + [h, k, g] + [k, g, h] - [k, h, g] \tag{5}$$

is a generic 3-cycle.

A *shuffle* of the string of letters $y_1, \ldots, y_r$ through the letters $x_1, \ldots, x_q$ is any sequence $z_1, \ldots, z_{q+r}$, consisting of the $q + r$ letters x_i, y_j in some order such that the x's alone and the y's alone appear in their given order. We define an operation $\overset{*}{0}$ on cells of $A^0(\Pi)$ as

$$[x_1, \ldots, x_q] \overset{*}{0} [y_1, \ldots, y_r] = \sum (-1)^e [z_1, \ldots, z_{q+r}], \tag{6}$$

the sum extending over all shuffles, with the sign of each shuffle determined by e, the number of pairs of indices (i, j) for which x_i follows y_j in the shuffle. With this notation, the generic cycles (4) and (5) may be expressed as $[g]\overset{*}{0}[h]$ and $[g]\overset{*}{0}[h]\overset{*}{0}[k]$, respectively. In general, we have

THEOREM 1. *If F_e is the free abelian group on generators $g_1, g_2, \ldots$, then for each dimension $q \geq 1$ the integral homology group $H_q(A^0(F_e^*))$ of the generic subcomplex is a free abelian group with the generators*

$$[g_{i_1}]\overset{*}{0}[g_{i_2}]\overset{*}{0} \cdots \overset{*}{0}[g_{i_q}], \qquad i_1 < i_2 < \ldots < i_q. \tag{7}$$

2. *The Bar Construction for Complexes*.—In general, a *product of excess* $k \geq 0$ in a complex L is a bilinear function $a_* b$ on the chains a, b of L such that, if $d(a)$ denotes the dimension of a, and if $d_k(a) = d(a) + k$,

$$d(a_* b) = d(a) + d(b) + k \quad \text{(i.e., } d_k(a_* b) = d_k a + d_k b) \tag{8}$$

$$a_*(b_* c) = (a_* b)_* c, \tag{9}$$

$$b_* a = (-1)^e a_* b, \qquad e = (d_k a)(d_k b), \tag{10}$$

$$\partial(a_* b) = (\partial a)_* (b) + (-1)^{d_k(a)} a_*(\partial b). \tag{11}$$

In particular, (6) defines a product of excess zero in $A^0(\Pi)$, for Π abelian.

Let L be an abstract cell complex with a product $\#$ of excess $k - 1$. Define a new complex $M = \mathfrak{B}(L)$ whose cells are all expressions

$$\sigma = [\alpha_1|\alpha_2| \ldots |\alpha_p], \qquad \alpha_i \text{ cells of } L, \tag{12}$$

where $|$ is short for $|_k$ (a k fold bar). This cell has dimension

$$d(\sigma) = d(\alpha_1) + \ldots + d(\alpha_p) + (p - 1)k$$

(each bar counts as k dimensions), and has boundary

$$\partial\alpha = \sum_{i=1}^{p} (-1)^{\epsilon_{i-1}} [\alpha_1| \ldots |\alpha_{i-1}|\partial\alpha_i|\alpha_{i+1}| \ldots |\alpha_p] +$$
$$\sum_{i=1}^{p-1} (-1)^{\epsilon_i}[\alpha_1| \ldots | \alpha_{i-1}|\alpha_i \# \alpha_{i+1}|\alpha_{i+2}| \ldots |\alpha_p] \tag{13}$$

where $\epsilon_i = d_k(\alpha_1) + \ldots + d_k(\alpha_i)$. In formula (13), if $\partial\alpha_i$ or $\alpha_i \# \alpha_{i+1}$ is not a cell but a chain, the term is to be expanded by linearity. One proves that $\partial\partial = 0$. Now define a new product $*$ in $\mathfrak{B}(L)$. For $\tau = [\beta_1| \ldots |\beta_q]$ and σ as in (12), set

$$\sigma_*\tau = \sum(-1)^{\epsilon}[\gamma_1| \ldots |\gamma_{p+q}]$$

with the sum taken over all shuffles $\gamma_1, \ldots, \gamma_{p+q}$ of the letters $\beta_1, \ldots, \beta_q$ through the letters $\alpha_1, \ldots, \alpha_p$. The sign ϵ of any shuffle is the sum $\sum d_k(\alpha_i)d_k(\beta_j)$, taken over all pairs of indices i, j, such that β_j comes before α_i in the shuffle. This new product has excess k in $\mathfrak{B}(L)$, and the new complex $M = \mathfrak{B}(L)$ contains L (as cells (12) with $p = 1$). Write $\mathfrak{B}(L, \#) = (M, *)$.

A complex L' with a product $\#'$ of excess $k - 1$ is said to be a *reduction* of the complex L with product $\#$ of excess $k - 1$ if there is a chain equivalence $f:L \to L'$ which maps L onto L' and which preserves products; i.e., is such that

$$f(a \# b) = (fa) \#' (fb) \tag{14}$$

for all chains a, b of L.

THEOREM 2.   *If $(L', \#')$ is a reduction of $(L, \#)$, then $\mathfrak{B}(L', \#')$ is a reduction of $\mathfrak{B}(L, \#)$.*

In fact, the new chain equivalence $\bar{f}:\mathfrak{B}(L, \#) \to \mathfrak{B}(L', \#')$ may be defined as

$$\bar{f}[\alpha_1|\alpha_2| \ldots |\alpha_p] = [f\alpha_1|f\alpha_2| \ldots |f\alpha_p]. \tag{15}$$

3. The Abelian Complex.—For any abelian group Π, we construct the "abelian" complex of Π, by starting with the complex $A^0(\Pi) = K(\Pi, 1)$ with the product $\overset{*}{0}$ of (6) and applying the bar construction repeatedly to obtain new complexes

$$(A^1(\Pi),\overset{*}{1}) = \mathfrak{B}(A^0(\Pi),\overset{*}{0}), (A^i(\Pi),\overset{*}{i}) = \mathfrak{B}(A^{i-1}(\Pi), \overset{*}{i-1}). \tag{16}$$

Let $A(\Pi) = \mathfrak{B}^{\infty}(A^0(\Pi), \overset{*}{\underset{0}{}})$ be the union of all these complexes. This complex $A(\Pi)$ has in dimensions 1 and 2 just the cells $[x]$ and $[x, y]$ of $A^0(\Pi)$. In dimension 3, there are the cells $[x, y, z]$ of $A^0(\Pi)$, plus the new cell $[x|y]$ of $A^1(\Pi)$, with

$$\partial[x|y] = [x, y] - [y, x]. \tag{17}$$

Thus the presence of this cell will make the generic cycle (4) a boundary. In dimension 4 there are the cells $[x, y, z, t]$, $[x, y|z]$, $[x|y, z]$, and $[x||y]$, with boundaries

$$\partial[x, y|z] = [\partial(x, y)|z] - [(x, y) \overset{*}{\underset{0}{}} z], \tag{18}$$

$$\partial[x|y, z] = [x|\partial(y, z)] + [x \overset{*}{\underset{0}{}} (y, z)], \tag{19}$$

$$\partial[x||y] = -[x \overset{*}{\underset{1}{}} y] = -[x|y] - [y|x]. \tag{20}$$

In general, any cell σ of $A(\Pi)$ is a string of elements $x_1, \ldots, x_r$ of Π, separated by commas or by bars of various multiplicities. For the case when Π is the free abelian group F_a, the cell σ is *generic* provided that the elements $x_1, \ldots, x_r \epsilon F_a$ which appear in σ are generic and pairwise disjoint. These generic cells again form a subcomplex $A(F_a^*)$.

THEOREM 3. *The generic abelian complex $A(F_a^*)$ has the integral homology groups*

$$H_1(A(F_a^*)) \cong F_a, \qquad H_q(A(F_a^*)) = 0, \qquad q > 1. \tag{21}$$

The proof depends on first using the result of Theorem 1 to reduce $A^0(F_a^*)$ to a simpler complex G_0^0 in which the cells correspond exactly to the cycles (7). The homology groups of $\mathfrak{B}(G_0^0)$ may then be determined, much as in (7). This gives a reduction G_1^1 of $\mathfrak{B}(G_0^0)$; the result follows by iteration of this process.

4. The Cubical Complex.—In our previous study[2] of the effect of a single homotopy group π_m of a space upon the homology groups of that space, we had occasion to introduce for any abelian group Π a complex $Q(\Pi)$ in which the cells were cubical arrays of elements of Π, together with a normalization subcomplex $Q_N(\Pi)$, consisting of "slabs" and "diagonals" in these cubical arrays. For any abelian coefficient group G, the cubical cohomology groups of Π were defined as the relative cohomology groups of the pair $Q(\Pi)$, $Q_N(\Pi)$.

For the free group F_a we may again introduce the generic complex $Q(F_a^*)$ and its normalization subcomplex $Q_N(F_a^*) = Q_N(F_a) \cap Q(F_a^*)$.

THEOREM 4. *The integral homology groups for the generic cubical complex are*

$$H_1(Q(F_a^*), Q_N(F_a^*)) \cong F_a, \qquad H_q(Q(F_a^*), Q_N(F_a^*)) = 0, \qquad q > 1. \tag{22}$$

In dimensions 1 and 2, $Q(\Pi)$ and $A(\Pi)$ are identical. This fact, plus the "generic acyclicity" expressed by (21) and (22), allows us to prove

THEOREM 5. *For any abelian group* Π, *the complex* $A(\Pi)$ *is chain equivalent to* $Q(\Pi)$ *modulo* $Q_N(\Pi)$.

Since this result still holds when $Q(\Pi)$ is replaced by any alternative construction enjoying the same formal properties (in particular, (22)), it follows that the homology and cohomology groups of $A(\Pi)$ depend not on the particular complex $A(\Pi)$, but essentially upon the fact that Π is an abelian group. We therefore call these groups the "abelian" homology and cohomology groups of Π; thus

$$A_q(\Pi) = H_q(A(\Pi)), \qquad A^q(\Pi; \ G) = H^q(A(\Pi); \ G) \qquad (23)$$

for any coefficient group G. The groups of the complex $A^l(\Pi)$ will also be called the abelian homology and cohomology groups of Π on *level l*, in symbols

$$A_q^l(\Pi) = H_q(A^l(\Pi)), \qquad A^{q, l}(\Pi; \ G) = H^q(A^l(\Pi); \ G). \qquad (24)$$

Among the cells of $A(\Pi)$ which are not in $A^l(\Pi)$ the cell $[x|_{l+1}y]$ has the lowest possible dimension $l + 3$. Hence the homology groups of $A^l(\Pi)$ agree with those of $A(\Pi)$ through dimension $l + 1$, so that

$$A_q^l(\Pi) \cong A^q(\Pi), \qquad A^{q, l}(\Pi; \ G) \cong A^q(\Pi; \ G), \qquad q \leqq l + 1. \qquad (25)$$

By Theorem 5, the abelian cohomology groups of Π are identical with the "cubical" cohomology groups of $Q(\Pi)$ modulo $Q_N(\Pi)$. We conjecture also that the levels of $A(\Pi)$ agree with the levels of $Q(\Pi)$, as introduced in our previous note,[2] so that

$$Q^{q, l}(\Pi; \ G) \cong A^{q, l}(\Pi; \ G). \qquad (26)$$

We have established this result for $q = 1, 2$, and 3. In view of the geometric interpretation of the Q groups in Theorem 3 of our previous note, we have:

THEOREM 6. *If* X *is an arcwise connected topological space with a given homotopy group* $\pi_m (m > 1)$ *and with all other homotopy groups trivial, the singular cohomology groups of* X *in dimensions between* m *and* $2m - 1$, *and with any coefficient group* G, *are determined by* π_m *according to isomorphisms:*

$$H^{m-1+k}(X; \ G) = A^k(\pi_m; \ G), \qquad k = 1, \ldots, m.$$

If the conjecture (26) holds, we would have a similar isomorphism for all k, in the form $H^{m-1+k}(X; \ G) = A^{k, m-1}(\pi_m; \ G)$. In view of (25), this agrees with the result of the Theorem for low k.

5. *Explicit Homology Groups.*—The complex $A(\Pi)$ and its reductions may be used for explicit computations, with the aid of Theorem 2.

Let $\Pi = J$ be an infinite cyclic group. The ordinary cohomology groups

$H^q(J, G)$ are zero for $q > 1$; in fact, the usual proof of this fact may be used to show that $A^0(J)$ can be reduced to the complex U^0 which has just one cell, a cell [1] of dimension 1, with boundary zero and product $[1] \overset{.}{*} [1]$ $= 0$ of excess zero. An infinite sequence of bar constructions on U^0 yields, as in (16), a complex U^∞, and Theorem 2 shows that $A(J)$ is chain equivalent to U^∞. The complex U^∞ has only a finite number of cells in each dimension; hence any homology group of U^∞ may be computed in a finite number of steps. For example, if (n) denotes a cyclic group of order n and $+$ a direct sum, one has

$$A_1(J) = (\infty), A_2(J) = 0, A_3(J) = (2), A_4(J) = 0, A_5(J) = (2) + (3),$$
$$A_6(J) = 0, A_7(J) = (2) + (2), A_8(J) = 0, A_9(J) = (2) + (2) + (3) + (5),$$
$$A_{10}(J) = (2), A_{11}(J) = (2) + (2) + (2).$$

Let $\Pi = J/hJ$ be a cyclic group of order h. The usual computation[s] of the ordinary cohomology groups $H^q(\Pi, G) = H^q(A^0(\Pi), G)$ proves $A^0(\Pi)$ reducible to the complex $M^0(h)$ which has one cell $\{n\}$ in each dimension $n > 0$, with the boundary formulas

$$\partial\{2m\} = h\{2m - 1\}, \qquad \partial\{2m + 1\} = 0,$$

and which has a product of excess zero defined, using binomial coefficients, as

$$\{2m\}_*\{2n\} = \binom{m + n}{n} \{2(m + n)\}, \qquad \{2m + 1\}_*\{2n + 1\} = 0,$$

$$\{2m\}_*\{2n + 1\} = \{2m + 1\}_*\{2n\} = \binom{m + n}{n} \{2(m + n) + 1\}.$$

Hence $A(J/hJ)$ is reducible to the complex $M^\infty(h)$ obtained from $M^0(h)$ by iterated bar construction. The latter complex again has but a finite number of cells in each dimension; explicit computation shows for instance that $A_6(J/hJ) = (3, h) + (2, h)$, where $(2, h)$ denotes a cyclic group of order the greatest common divisor of 2 and h.

If L and L' are complexes with products $\#$, $\#'$ of excess zero, one may form an "extended" *tensor product* complex $L \square L' = L \cup L' \cup (L \otimes L')$ in which the cells are cells σ of L, cells σ' of L', and new cells $\sigma \otimes \sigma'$ with dimension $d(\sigma) + d(\sigma')$. It is convenient to write $\sigma = \sigma \otimes \theta$, $\sigma' = \theta \otimes \sigma'$, where θ is the "void" cell with boundary 0. The boundary formulas are

$$\partial(\sigma \otimes \sigma') = (\partial\sigma) \otimes \sigma' + (-1)^{d(\sigma)}\sigma \otimes (\partial\sigma') \qquad (27)$$

and the product (with the conventions $\sigma \# \theta = \sigma$, $\theta \# \sigma' = \sigma'$) is

$$(\sigma \otimes \sigma')\#(\tau \otimes \tau') = (-1)^{d(\sigma')d(\tau)}(\sigma \# \tau) \otimes (\sigma' \#' \tau') \qquad (28)$$

THEOREM 7. *If K is reducible to L and K' to L', then $K \,\square\, L$ is reducible to $K' \,\square\, L'$.*

THEOREM 8. *For a direct sum $\Pi_1 + \Pi_2$ of abelian groups Π_1 and Π_2, $A^0(\Pi_1 + \Pi_2)$ is reducible to $A^0(\Pi_1) \,\square\, A^0(\Pi_2)$.*

Together with Theorem 2 and the results for cyclic groups, these Theorems prove

THEOREM 9. *If Π is a finitely generated abelian group, then any abelian homology or cohomology group $A_e(\Pi)$ or $A^e(\Pi, J)$ may be computed in a finite number of steps.*

In certain cases, the results can be put into invariant form. Thus for any abelian group Π (not necessarily finitely generated) we have

$$A_1(\Pi) \cong \Pi, \qquad A_2(\Pi) = 0, \qquad A_3(\Pi) \cong \Pi/2\Pi, \qquad A_4(\Pi) \cong {}_2\Pi, \qquad (29)$$

where $_2\Pi$ denotes the subgroup of all elements of order 2 in Π.

In this sense, the theory of generic acyclicity provides explicit methods for the computation of certain homology groups of the complex $K(\Pi, m)$ which gives the effect of the mth homotopy group of a space upon homology. If conjecture (26) holds, these results will apply to all homology groups of $K(\Pi, m)$.

* Essential portions of the study here summarized were done during the tenure of a John Simon Guggenheim Fellowship by one of the authors.
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[2] Eilenberg, S., and MacLane, S., PROC. NATL. ACAD. SCI., 36, 443–447 (1950).
[3] Eilenberg, S., *Bull. Am. Math. Soc.*, 55, 3–37 (1949), especially p. 22.

ARCS IN LOCALLY COMPACT GROUPS

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We outline the proof of a theorem useful in the topological investigation of groups: namely, that every locally compact group which is not totally disconnected contains an arc. With the aid of this theorem we are able to prove that every finite dimensional, locally compact group contains a one-parameter subgroup. These results accord with the conjecture that every locally compact group is a generalized Lie group.[1]

The results on finite dimensional groups were largely inspired by the work of Deane Montgomery.

1. The Topology of the Compact Subsets of a Locally Compact Space.— Let T be a locally compact space whose topology is defined by a fixed

uniform structure. We shall assume throughout that all uniformities are symmetrical. Let $\mathfrak{C}$ be the family of all compact subsets of T. For each uniformity α of T we can define a uniformity α' of $\mathfrak{C}$ by setting $(A, B) \epsilon \alpha'$ if and only if (i) for each $a \epsilon A$, there exists $b \epsilon B$ such that $(a, b) \epsilon \alpha$ and (ii) for each $b \epsilon B$, there exists $a \epsilon A$ such that $(a, b) \epsilon \alpha$. It is easily verified that this family of uniformities determines a separated uniform structure for $\mathfrak{C}$; hence $\mathfrak{C}$ becomes a completely regular topological space. This definition is applicable to the family of all closed subsets of any uniform space, and in this form it becomes a generalization of the well-known topology of the closed subsets of a metric space. The space $\mathfrak{C}$ is compact or locally compact according as T is compact or locally compact, and it turns out that the topology of $\mathfrak{C}$ is independent of the choice of uniform structure for T. (This would be false if T were not locally compact.)

LEMMA 1. *Let $\mathfrak{A}$ be a family of compact subsets of T which is linearly ordered by inclusion and, as a subset of $\mathfrak{C}$, is closed and connected. Let $B =$ $\cap A \epsilon \mathfrak{A}$. If B is connected then every set $A \epsilon \mathfrak{A}$ is connected.*

2. *Semigroups.*—By a semigroup G we shall mean an algebraic system with an associative binary operation and a unit element e. We write the operation by juxtaposition. In a semigroup it may happen that we can solve the equation $ax = e$ for some $a \neq e$. If this is the case for all a we have a group; however, we shall be interested in the opposite case. Suppose that from $a = axy$ follows $a = ax$. We shall say that such a semigroup is ordered because it is partially ordered by the relation $\geq$ where $a \geq b$ means that $a = bx$ for some $x \epsilon G$. If S is a subsemigroup of an ordered semigroup G, then S is also ordered, but the ordering of S need not coincide with the ordering of G.

If G is also endowed with a Hausdorff topology such that the mapping $(a, b) \to ab$ of $G \times G$ into G is continuous, we say that G is a topological semigroup. Suppose that the topology of G is defined by a uniform structure. We shall say that the uniform structure is right invariant if, for every uniformity α, from $(a, b) \epsilon \alpha$ follows $(ac, bc) \epsilon \alpha$. A subset L of a topological semigroup is called a local subsemigroup if $e \epsilon L$ and, for some neighborhood V of e in L, $VV \subset L$. The notions of local semigroup and local isomorphism are defined similarly following the usual definitions for groups.

LEMMA 2. *Let G be a locally compact, ordered semigroup. Assume that G has a right invariant uniform structure with a countable base. Let U be a compact neighborhood of the identity in G. Suppose that we can find sequences $x_1, x_2, \ldots$ of elements of G and $n_1, n_2, \ldots$ of integers such that $x_i \to e$ and $x_i^{n_i} \epsilon' U$. Then G contains a compact, connected, linearly ordered local subsemigroup L which contains more than one element.*

If A_i is the compact set $\{e, x_i, x_i^2, \ldots, x_i^{n_i - 1}\}$, then any limit (in the sense

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of §1) of the sequence A_i satisfies the requirements for L. The requirement of countability is not actually necessary, but the proof becomes rather involved without it.

LEMMA 3. *Let L be a compact, connected, linearly ordered local semigroup with more than one element. Either L is locally isomorphic to the additive semigroup of non-negative real numbers or every neighborhood of e contains another idempotent.*

3. *Application to Groups.*—Let G be a locally compact group, and let $\mathfrak{C}$ be the family of its compact subsets. $\mathfrak{C}$ is a semigroup under multiplication in G, the unit element being the set $\{e\}$. Starting with the right invariant uniform structure of G, we define a uniform structure and topology for $\mathfrak{C}$ as in §1. It turns out that the uniform structure of $\mathfrak{C}$ is right invariant and that multiplication in $\mathfrak{C}$ is continuous. Let $\mathfrak{S}$ be the subsemigroup of those compact sets which contain e. $\mathfrak{S}$ is closed in $\mathfrak{C}$, so it is itself a locally compact semigroup. Furthermore, since $AB \supset A$ for A, $B \in \mathfrak{S}$, it is clear that $\mathfrak{S}$ is ordered.

Now suppose that G is metrizable and connected and contains more than one point. Let U be a compact neighborhood of e in G which does not contain all of G. Let $\mathfrak{U}$ be the family of compact subsets of U containing e. Then $\mathfrak{U}$ is a compact neighborhood of $\{e\}$ in $\mathfrak{S}$. Let X_i be a fundamental sequence of compact neighborhoods of the identity in G; then $X_i \to \{e\}$ in $\mathfrak{S}$. Since G is connected, for each i there is an integer n_i such that $X_i^{n_i} \in' \mathfrak{U}$. All the conditions of Lemma 2 are satisfied by $\mathfrak{S}$; hence there exists in $\mathfrak{S}$ a compact, connected, linearly ordered local subsemigroup $\mathfrak{L}$ whose structure is given by Lemma 3. If idempotents appear in $\mathfrak{L}$, they are compact subgroups of G. All the elements of $\mathfrak{L}$ are connected subsets of G by Lemma 1. This proves

LEMMA 4. *Let G be a connected, locally compact, metrizable group containing more than one point. Either G contains arbitrarily small connected, compact subgroups larger than $\{e\}$ or there exists a family $F(t)$ $(0 \leq t \leq 1)$ of connected, compact subsets of G such that (i) $F(t) \neq e$ if $t > 0$, (ii) $\cap_{t > 0}$ $F(t) = F(0) = \{e\}$ and (iii) $F(t)F(u) = F(t + u)$ if $t + u \leq 1$.*

If G has finite dimension, then an infinite decreasing sequence of connected subgroups cannot exist,[2] so the second alternative must hold. Although we use it only to prove Theorem 1 below, the family $F(t)$ seems to be of considerable interest in itself. In a Lie group, the sets $F(t)$ can be chosen so that they are all neighborhoods of the identity $(t > 0)$. This is presumably possible for any locally connected group of finite dimension, but the author cannot prove it without assumptions of an analytic character.

THEOREM 1. *Every connected, locally compact group which contains more than one element contains an arc.*

If G has a family of sets $F(t)$ as described in Lemma 4, we can construct

an arc in the same way that one constructs an isometric arc in a convex metric space. If G contains a compact, connected subgroup, then it contains even a one-parameter subgroup. There remains only the non-metrizable case. Here G has a compact normal subgroup N such that G/N is metrizable. Either N contains an arc or N is totally disconnected and we can "raise" an arc of G/N.

The set of elements which can be joined to the identity by an arc in G is a normal subgroup $G\dagger$. It is a reasonable conjecture that $G\dagger$ is dense. One could prove this by showing that an arc of $G/G\dagger^-$ could be "raised" into G.

4. *Finite Dimensional Groups.*—LEMMA 5. *Let X be a compact set of dimension r in a locally arcwise-connected metric group. Then either X has an interior point or there exists an arc A such that dim $(AX) > r$.*

This lemma is essentially due to Montgomery.[2] Our proof, which uses homotopy theory, follows the intuition closely.

THEOREM 2. *Let G be a locally arcwise-connected group of dimension n. Then any compact subset of G whose dimension is n has an interior point.*

THEOREM 3. *Let G be a locally arcwise-connected group of finite dimension. Then G is locally compact.*

In particular, a locally connected, complete, metric group of finite dimension is locally compact.

We return to the consideration of $G\dagger$, the arcwise-connected subgroup of G. We can define a new and stronger topology for G under which G becomes locally arcwise-connected and $G\dagger$ becomes the connected component of the identity: If V is a neighborhood of the identity in G, then the arcwise-connected component of the identity in V is taken as a new neighborhood. We shall denote by G^* the group $G\dagger$ taken with this new topology. If G is metrizable, so is G^*. If G has finite dimension, then so does G^* because it has a faithful representation in G. In this case G^* is locally compact by Theorem 3; in general, however, G^* need not be locally compact.

These considerations apply to any arcwise connected subgroup of a finite dimensional group and give

THEOREM 4. *Any arcwise connected subgroup of a Lie group is analytic.*

LEMMA 6. *A locally connected group of finite dimension greater than one contains a proper connected closed subgroup.*

This lemma has been proved by Montgomery for locally Euclidean groups.[3] Our argument follows his using Theorem 2 in place of the Brouwer theorem of invariance of domain.

THEOREM 5. *Every connected, locally compact group of finite positive dimension contains a one-parameter subgroup.*

The proof is by induction on the dimension n of G. If $n = 1$, then G^* is locally arcwise-connected and of dimension 1. By Theorem 2 it

must be locally Euclidean and itself a one-parameter group. Then G^* determines a one-parameter subgroup of G. Suppose that $k > 1$ and that the theorem is true for $n < k$. If G has dimension k and is locally connected, then we can find a one-parameter subgroup of the subgroup guaranteed by Lemma 6, whose dimension is positive and less than k. Finally suppose that G has dimension k but is not locally connected. Then G^* is a locally compact, locally connected group of positive dimension at most k, so G^* contains a one-parameter subgroup, which in turn gives us a one-parameter subgroup of G.

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PSEUDO-CONFORMAL GEOMETRY OF POLYGENIC FUNCTIONS OF SEVERAL COMPLEX VARIABLES

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1. A complex function

$$w = F(z^\alpha) = F(z^1, \ldots, z^n) = \phi(x^\alpha; y^\alpha) + i\psi(x^\alpha; y^\alpha), \qquad (1)$$

where the real ϕ and ψ are single-valued continuous functions and possess continuous partial derivatives over a region R of $2n$ dimensional real space Σ_{2n} of coordinates $(x^1, \ldots, x^n; y^1, \ldots, y^n)$ is termed a *polygenic function*[1] of the n complex variables $z^\alpha = x^\alpha + iy^\alpha$, where $\alpha = 1, \ldots, n$.

We shall study the first derivatives of a polygenic function and also present some new results in pseudo-conformal geometry.

2. For the class of polygenic functions, the linear operators

$$\frac{\partial}{\partial z^\alpha} = \frac{1}{2}\left(\frac{\partial}{\partial x^\alpha} - i\frac{\partial}{\partial y^\alpha}\right); \quad \frac{\partial}{\partial \bar{z}^\alpha} = \frac{1}{2}\left(\frac{\partial}{\partial x^\alpha} + i\frac{\partial}{\partial y^\alpha}\right), \qquad (2)$$

are important. These are called the *mean* and *phase derivatives*, respectively. The operations $\dfrac{\partial}{\partial z^\alpha}$ and $\dfrac{\partial}{\partial \bar{z}^\alpha}$ are not partial derivatives but signify the application of the linear operators (2).

3. A polygenic function w is monogenic over R if and only if

$$\frac{\partial w}{\partial \bar{z}^{\alpha}} = 0, \qquad \text{where } \alpha = 1, \ldots, n. \tag{3}$$

These are equivalent to the $2n$ Cauchy-Riemann equations. The real and imaginary parts obey the n^2 Poincaré partial differential equations of second order

$$\frac{\partial^2 w}{\partial \bar{z}^{\alpha} \partial \bar{z}^{\beta}} = 0, \qquad \text{where } \alpha, \beta = 1, \ldots, n. \tag{4}$$

A *multiharmonic function* is any solution w of this Poincaré system.[2] If $w = \phi + i\psi$ is monogenic over R, then ϕ and ψ are *conjugate-multiharmonic* over R.

4. The locus of points in Σ_{2n}, defined by the equations

$$z^{\alpha} = f^{\alpha}(Z^{\beta}), \tag{5}$$

where the n f^{α} are monogenic functions over a region of the $2m$ dimensional parametric space Σ'_{2m} of the m complex variables $Z^{\beta} = X^{\beta} + iY^{\beta}$, where $\beta = 1, \ldots, m$, such that the jacobian-matrix

$$\left(\frac{\partial x^{\alpha}}{\partial X^{\beta}}, \frac{\partial x^{\alpha}}{\partial Y^{\gamma}}; \frac{\partial y^{\alpha}}{\partial X^{\beta}}, \frac{\partial y^{\alpha}}{\partial Y^{\gamma}} \right), \tag{6}$$

is of rank $2m$, is called a *pseudo-conformal manifold* Σ_{2m} of $2m$ dimensions. Evidently $m \leqq n$.

If $m = n$, there results a correspondence, called a *pseudo-conformal transformation* T, between the two pseudo-conformal manifolds Σ_{2n} and Σ'_{2n}. All such maps T form the *pseudo-conformal group* G. The associated geometry is termed *pseudo-conformal geometry.*

Under G, any Σ_{2m} becomes a Σ_{2m}. For $m = 1$, any Σ_2 is called a *conformal or analytic surface.* The reason for this terminology is that G induces conformality between pairs of analytic surfaces.

The angle λ_{α} between any direction at a fixed point P on a conformal surface S and its orthogonal projection on the z^{α}-plane depends only on the position of the point P on S. Moreover the sum of the squares of the cosines of the resulting n angles $\lambda_1, \ldots, \lambda_n$, at P, is unity.[3]

An area A on a conformal surface S is the sum of the projected areas A_{α} on the z^{α}-planes.

5. Consider a polygenic function w defined over a conformal surface S in R. If $Z = X + iY$ is the parameter describing S, then

$$\frac{dw}{dZ} = \left(\frac{\partial w}{\partial z^{\alpha}} \frac{dz^{\alpha}}{dZ} \right) + \left(\frac{\partial w}{\partial \bar{z}^{\alpha}} \frac{d\bar{z}^{\alpha}}{d\bar{Z}} \right) e^{-2i\theta}, \tag{7}$$

in which the repeated index α means to sum with respect to that index from 1 to n. It is noted that θ is the angle between a direction at a point P on S and the parametric curve $Y =$ const., through P on S. Representing dw/dZ in a plane, called the *derivative plane* Δ, dw/dZ is depicted as a clock[4] with center vector $H + iK = \dfrac{\partial w}{\partial z^\alpha} \dfrac{dz^\alpha}{dZ}$, and phase vector $h + ik = \dfrac{\partial w}{\partial \bar{z}^\alpha} \dfrac{d\bar{z}^\alpha}{dZ}$. This clock depends on the point P, the conformal surface S through P, and the parameter Z describing S.

If a change of the parameter Z is performed, the central and phase vectors of the clock Γ are multiplied by the same real number $\rho > 0$ and rotated through equal angles but in opposite directions. Denote this operation on a clock Γ by $S^*(\Gamma)$. Then all the clocks corresponding to a fixed point P and a fixed surface S through P are the ∞^2 clocks $S^*(\Gamma)$.

The totality of clocks at a given point P is ∞^{2n} but essentially there are ∞^{2n-2}, one to each conformal surface S through P.

If $\Gamma_1, \ldots, \Gamma_n$ are n clocks belonging to n distinct conformal surfaces S_1, $\ldots, S_n$, not all contained in the same Σ_{2n-2}, then any clock Γ at P is given by

$$\Gamma = S_1^*(\Gamma_1) + \ldots + S_n^*(\Gamma_n). \tag{8}$$

Thus the totality of clocks at a given point P forms an abstract vector space of n dimensions.

A set of m clocks $\Gamma_1, \ldots, \Gamma_m$, at a given point P, is found to be linearly dependent if and only if they are obtained from m conformal surfaces S_1, $\ldots, S_m$, through P, all of which belong to the same pseudo-conformal manifold Σ_{2m-2}.

A polygenic function w is monogenic at a given point if and only if dw/dZ is unique along each of n distinct conformal surfaces through P, not all contained in the same Σ_{2n-2}.

This result generalizes the usual definition of a monogenic function.

A polygenic function w is multiharmonic in Σ_{2n} if and only if it is multiharmonic over every Σ_{2m} where m is fixed and $m \leq n$.

Also it may be shown that the center transformation is direct conformal[*] for every S if and only if w is multiharmonic in Σ_{2n}.

6. The *pseudo-angle* between a $(2n - 1)$ dimensional manifold S_{2n-1}: $F(x^1, \ldots, x^n; y^1, \ldots, y^n) = 0$, and a curve $Cx^\alpha: = x^\alpha(t)$, where $\alpha = 1, \ldots, n$, at a given point P of intersection, is

$$\theta = \text{arc tan} \dfrac{\dfrac{\partial F}{\partial x^\alpha} dx^\alpha + \dfrac{\partial F}{\partial y^\alpha} dy^\alpha}{-\dfrac{\partial F}{\partial y^\alpha} dx^\alpha + \dfrac{\partial F}{\partial x^\alpha} dy^\alpha}. \tag{9}$$

The pseudo-angle characterizes the pseudo-conformal group G.[5]

Let S_{2m} be a fixed $2m$ dimensional manifold contained in Σ_{2n} so that $m \leq n$. Let P be an arbitrary point of S_{2m}. Suppose that S_{2n-1} is an arbitrary $(2n - 1)$ dimensional manifold in Σ_{2n}, which contains P, and let the intersection of S_{2m} and S_{2n-1} be an S_{2m-1}. Thus S_{2m} is not contained in S_{2n-1}.

The pseudo-angle between any curve C, in S_{2m}, and S_{2n-1} at P, is equal to that between C and S_{2m-1}, for every S_{2n-1}, if and only if S_{2m} is pseudo-conformal.

This gives a geometric characterization of the pseudo-conformal manifolds Σ_{2m} contained in a given pseudo-conformal manifold Σ_{2n}, where $m \leq n$.

If $w = \phi + i\psi$ is monogenic over R, then the curves of $\phi =$ const. are pseudo-orthogonal to the manifolds $\psi =$ const.

For $n = 1$, this reduces to the well-known result that the components of a monogenic function of a complex variable give rise to an orthogonal isothermal net.

[1] The term polygenic was introduced by Kasner in 1927. See: "A New Theory of Polygenic (or non-Monogenic) functions," *Science*, 66, 581–582 (1927). The term non-analytic is also used.

[2] Poincaré, *Compt. rend.*, 96, 238, (1883); *Acta Math.*, 2, 99, (1883), 22, 112 (1898); *Palermo Rendiconti* (1907).

[3] Kasner, "Conformality in Connection with Functions of Two Complex Variables," *Trans. Am. Math. Soc.*, 48, 50–62 (1940).

[4] Kasner and De Cicco, "The Geometry of Polygenic Functions," *Rev. Math. Univ. Tucuman (Argentina)*, 4, 7–45 (1944)

[5] De Cicco, "The Pseudo-Angle in Space of $2n$ Dimensions," *Bull. Am. Math. Soc.*, 51, 162–174 (1945). Also "Functions of Several Complex Variables and Multiharmonic Functions," *Am. Math. Monthly*, 56, 315–325 (1949).

THE ELEMENT OF VOLUME OF THE ROTATION GROUP

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The n-dimensional rotation group is an $\dfrac{n(n - 1)}{2}$-parameter group and if we set $n = 2k$ or $n = 2k + 1$, according as n is even or odd, it is usually convenient to adopt as k of these parameters the k angles $\theta_1, \theta_2, \ldots, \theta_k$ which determine the class of the group to which the particular element X of the group which we wish to specify belongs (the function of the remain-

ing $\dfrac{n(n-1)}{2} - k$ parameters being, then, to locate the element X in its

class). The element of volume of the rotation group appears, when this is done, as a product of two factors in which one of the factors involves only the parameters $\theta_1, \ldots, \theta_k$ (which serve to specify the class) and their differentials and the other factor involves the remaining $\dfrac{n(n-1)}{2} - k$ parameters and their differentials. We shall refer to the first of these factors as the class factor (the other being the non-class factor). Whenever the function which we wish to integrate over the group is a class function it is sufficient to know the class factor of the element of volume of the group (the other factor canceling out in the process of averaging over the group) and, so far as we know, it is only the class factor of the element of volume of the n-dimensional rotation group that has been furnished to date. We propose to give in the present note the complete expression of the element of volume of the n-dimensional rotation group. The calculations necessary to furnish this yield at the same time the element of volume of the n-dimensional Lorentz group and of the n-dimensional quasi-Lorentz groups. The parameters we shall use are the analogs for the n-dimensional rotation group of the familiar Eulerian angles ϕ, θ, ψ (for the 3-dimensional rotation group); these have certain advantages (in the physical applications) over the class and non-class parameters (which are, from the theoretical point of view, more attractive) and the element of volume of the 3-dimensional rotation group in terms of the Eulerian angles, namely, $\sin\theta\, d\phi\, d\theta\, d\psi$ is already well known and widely used. The progress made in the present note is the extension of this result to the general value of n; the case $n = 4$ is of particular interest in view of the intimate connection between the 4-dimensional rotation group and the 4-dimensional Lorentz group.

Any n-dimensional rotation matrix X may be written as the product of $\dfrac{n(n-1)}{2}$ plane rotation matrices. We indicate by subscripts the plane in which the rotation takes place; thus $R_{12}(\phi)$ denotes the rotation $n \times n$ matrix of which the elements in the first two rows and first two columns are $\begin{pmatrix} \cos\phi & -\sin\phi \\ \sin\phi & \cos\phi \end{pmatrix}$, the remaining elements being zero, save the diagonal elements which are 1. Of the $\dfrac{n(n-1)}{2}$ angles involved, one in each of the plane rotation matrices whose product is X, $n-1$ are longitude (or equatorial) angles $\phi_1, \phi_2, \ldots, \phi_{n-1}$, each of which lies in the interval $-\pi \leq \phi < \pi$ while the remainder are latitude (or meridian) angles $\theta_1, \theta_2, \ldots, \theta_{(n-1)(n-2)/2}$ each of which lies in the interval $0 \leq \theta \leq \pi$. In the cases $n = 3, 4$ and 5, for example, we have

$X = R_{12}(\phi_2)R_{23}(\theta_1)R_{12}(\phi_1);$ $n = 3;$

$X = R_{12}(\phi_3)R_{23}(\theta_3)R_{34}(\theta_2)R_{12}(\phi_2)R_{23}(\theta_1)R_{12}(\phi_1);$ $n = 4;$

$X = R_{12}(\phi_4)R_{23}(\theta_6)R_{34}(\theta_5)R_{45}(\theta_4)R_{12}(\phi_3)R_{23}(\theta_3)R_{34}(\theta_2)R_{12}(\phi_2)R_{23}(\theta_1)R_{12}(\phi_1);$
     $n = 5.$

It helps in the calculations to observe that, except for the change in the dimensions of the plane rotation matrices involved, each of these expressions involves the preceding expression as a factor. Thus if we denote, for a moment, the typical element of the n-dimensional rotation group by X_n, so that $X_2 = R_{12}(\phi_1)$, X_3 is the product of X_2 (increased to dimension 3) by $R_{12}(\phi_2)R_{23}(\theta_1)$. Similarly X_4 is the product of X_3 (increased to dimension 4) by $R_{13}(\phi_3)R_{23}(\theta_3)\; R_{34}(\theta_2)$, X_5 is the product of X_4 (increased to dimension 5) by $R_{12}(\phi_4)R_{23}(\theta_6)R_{34}(\theta_5)R_{45}(\theta_4)$ and so on. The net result of this is that the element of volume of the n-dimensional rotation group involves the element of volume of the $(n - 1)$-dimensional rotation group as a factor. Thus the element of volume of the 2-dimensional rotation group is $d\phi_1$; the element of volume of the 3-dimensional rotation group is the product of this by $\sin \theta_1\, d\theta_1\, d\phi_2$, namely, $\sin \theta_1\, d\phi_1\, d\theta_1\, d\phi_2$. The element of volume of the 4-dimensional rotation group is the product of the element of volume of the 3-dimensional rotation group by $\sin^2 \theta_2 \sin \theta_3\, d\theta_2\, d\theta_3\, d\phi_3$, namely, $\sin \theta_1 \sin^2 \theta_2 \sin \theta_3\, d\phi_1\, d\theta_1\, d\phi_2\, d\theta_2\, d\theta_3\, d\phi_3$. Similarly, the element of volume of the 5-dimensional rotation group is the product of the element of volume of the 4-dimensional rotation group by $\sin^3 \theta_4 \sin^2 \theta_5 \sin \theta_6\, d\theta_4\, d\theta_5\, d\theta_6\, d\phi_4$ and so on. The final result may, therefore, be stated as follows:[1]

The element of volume of the n-dimensional rotation group is obtained by multiplying the product of the differentials of the $\dfrac{n(n - 1)}{2}$ angles ϕ and θ by the product of $n - 2$ factors of which the first is $\sin \theta_1$, the second $\sin^2 \theta_2$ sin θ_3, the third $\sin^3 \theta_4 \sin^2 \theta_5 \sin \theta_6$, and so on, the last being $\sin^{n-2} \theta_p$ $\sin^{n-3} \theta_{p+1} \ldots \sin \theta_{p + n - 3}$ where $p = \dfrac{(n - 2)(n - 3)}{2} + 1$ so that $p + n - 3 = \dfrac{(n - 1)(n - 2)}{2}.$

For the case of the Lorentz group $\sin \theta_{n-2}$ must be replaced by $\sinh \theta_{n-2}$, the range of θ being over all non-negative values. Thus the element of volume of the 4-dimensional Lorentz group is $\sin \theta_1 \sinh^2 \theta_2 \sin \theta_3\, d\phi_1\, d\theta_1\, d\phi_2\, d\theta_2\, d\theta_3\, d\phi_3$, the parametric space being furnished by the formulas $-\pi \le \phi_1 < \pi;\; 0 \le \theta_1 \le \pi;\; -\pi \le \phi_2 < \pi;\; 0 \le \theta_2;\; 0 \le \theta_3 \le \pi;\; -\pi \le \phi_3 < \pi$. Similarly, for the 4-dimensional quasi-Lorentz group, the element of volume is $\sin \theta_1 \sinh^2 \theta_2 \sinh \theta_3\, d\phi_1\, d\theta_1\, d\phi_2\, d\theta_2\, d\theta_3\, d\phi_3$ where, now, both θ_2 and θ_3 range over all positive values.

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[1] Details of the calculations will appear in *"Lectures on Matrices and Matrix Groups"* to be published by the Centro de Pesquisas Físicas, Rio de Janeiro, Brazil.

SCHWARZ' INEQUALITY AND LORENTZ SPACES

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The most fundamental formula in the theory of Euclidean or unitary metric spaces is, doubtless, Schwarz' inequality which states that the modulus of the scalar product of two vectors, real or complex, is dominated by the product of their magnitudes (the modulus of the scalar product being equal to the product of the magnitudes when, and only when, the two vectors are linearly dependent). Expressed in matrix notation $(x^*y)(y^*x) \leqslant (x^*x)(y^*y)$ where x and y are any two $n \times 1$ matrices, real or complex, and x^*, y^* are the conjugate transposed matrices of x and y, respectively. The proof, now classical, of this fundamental result runs as follows. If λ is any complex number the squared magnitude of $\lambda x + y$ is never negative; on varying the argument of λ it follows that neither of the two quadratic polynomial functions of $|\lambda|$: $(x^*x)|\lambda|^2 \pm 2|x^*y||\lambda| + (y^*y)$ is ever negative. Hence the quadratic polynomial function $(x^*x)\epsilon^2 + 2|x^*y|\epsilon + (y^*y)$ of the real variable ϵ is never negative and this implies, on using the theory of the zeros of a quadratic polynomial, Schwarz' inequality. We wish to point out in the present note that the positiveness of x^*x and the theory of the zeros of a quadratic polynomial are really somewhat foreign to Schwarz' fundamental inequality and to give the analog of Schwarz' inequality for Lorentz spaces, in which the scalar product of a non-zero $n \times 1$ matrix by itself may be negative or zero, instead of being always positive as in Euclidean or unitary spaces.

Let x_1 and x_2 be any two linearly independent $n \times 1$ matrices, real or complex, and denote by X the $n \times 2$ matrix whose column matrices are x_1 and x_2. If x_1 and x_2 are linear combinations of two other $n \times 1$ matrices, y_1 and y_2 (necessarily linearly independent), we have $X = YA$ where Y is the $n \times 2$ matrix whose column matrices are y_1 and y_2 and A is a non-singular 2×2 matrix. We take y_1 to be a multiple of x_1, so chosen that $y_1^*y_1 = 1$ and we set $y_2 = -(y_1^*x_2)y_1 + x_2$. Then $y_1^*y_2 = 0$ and y_2 is not the zero matrix. The 2×2 matrix Y^*Y, being the matrix $\begin{pmatrix} 1 & 0 \\ 0 & y_2^*y_2 \end{pmatrix}$, has a positive determinant and, since $X^*X = A^*(Y^*Y)A$, the determinant of the 2×2 matrix X^*X, being the product of the determinant of the 2×2 matrix Y^*Y by $|\det A|^2$, is positive. Since X^*X is the matrix $\begin{pmatrix} x_1^*x_1 & x_1^*x_2 \\ x_2^*x_1 & x_2^*x_2 \end{pmatrix}$ this proves Schwarz' inequality in the case where the two $n \times 1$ matrices x_1 and x_2 are linearly independent. When they are linearly dependent X may be written in the form YA where the 2×2

matrix A is singular. Hence the 2×2 matrix X^*X is singular; this completes the proof of Schwarz' inequality.

Before passing to the case of Lorentz spaces we make the obvious remark that if we wish to avail ourselves of the diagonalization property of Hermitian matrices the above proof of Schwarz' inequality may be given in the following (less elementary and only slightly more concise) variant form. The 2×2 matrix X^*X is Hermitian and so there exists a 2×2 unitary matrix U such that $U^*(X^*X)U$ is diagonal. Since X^*X is positively definite the diagonal elements of this diagonal form of X^*X (i.e., the characteristic numbers of X^*X) are positive and hence the determinant of X^*X is positive. This proves the part of Schwarz' inequality which is concerned with linearly independent $n \times 1$ matrices x_1 and x_2. The simplest and most direct proof of the part which is concerned with linearly dependent $n \times 1$ matrices x_1 and x_2 is that given above.

Turning now to the case of Lorentz spaces, let F be the diagonal $n \times n$ matrix all of whose diagonal elements are 1 save the last which is -1. The Lorentz scalar product of two $n \times 1$ matrices x and y is y^*Fx and we say that the $n \times 1$ matrix x, real or complex, is time-like, null or space-like according as x^*Fx is negative, zero or positive, respectively. The last element of a time-like $n \times 1$ matrix cannot be zero and it follows that every pair of linearly independent $n \times 1$ matrices x_1 and x_2 contains a space-like linear combination; this implies that if $x_1^*Fx_1 \leqslant 0$ and $x_1^*Fx_2 = 0$ then $x_2^*Fx_2 > 0$. Let us denote by X the $n \times 2$ matrix whose column matrices are any given pair of linearly independent matrices x_1 and x_2; if x_1 and x_2 are linear combinations of two other $n \times 1$ matrices y_1 and y_2, necessarily linearly independent, we have $X = YA$ where Y is the $n \times 2$ matrix whose column matrices are y_1 and y_2 and A is a non-singular 2×2 matrix. Hence the determinant of the 2×2 matrix X^*FX has the same sign as the determinant of the matrix Y^*FY and, if x_1 and x_2 are linearly dependent, the matrix X^*FX is singular. Taking x_1 and x_2 to be linearly independent there exists a linear combination y_1 of x_1 and x_2 which is such that $y_1^*Fy_1 = 1$ (since there exists a space-like linear combination of x_1 and x_2). We may suppose that y_1 is not a multiple of x_2 (since it is not a multiple of both x_1 and x_2) and we set $y_2 = -(y_1^*Fx_2)y_1 + x_2$. Then $y_1^*Fy_2 = 0$ and the determinant of the 2×2 matrix Y^*FY is $y_2^*Fy_2$. Thus, if there exists no time-like linear combination of x_1 and x_2, the determinant of the 2×2 matrix X^*FX is non-negative. Similarly, if there does exist a time-like linear combination of x_1 and x_2, this determinant is negative. Thus we have the following analog, for Lorentz spaces, of Schwarz' inequality:

$$(x_1^*Fx_1)(x_2^*Fx_2) < |x_1^*Fx_2|^2$$

if, and only if, there exists a time-like linear combination of the two $n \times 1$ matrices x_1 and x_2, supposed linearly independent.

Since there always exists a space-like linear combination of two linearly independent $n \times 1$ matrices, x_1 and x_2, one of the two characteristic numbers of the 2×2 Hermitian matrix X^*FX is positive. The second characteristic number may be either positive, zero or negative; in the first case, i.e., when the 2×2 Hermitian matrix X^*FX is positively definite, we say that the $n \times 2$ matrix X is space-like (so that every non-trivial linear combination of the column matrices of a space-like $n \times 2$ matrix is space-like). Thus we may complete the analog, for Lorentz spaces, of Schwarz' inequality as follows:

If $(x_1^*Fx_1)(x_2^*Fx_2) = |x_1^*Fx_2|^2$ there exists a null non-trivial linear combination of x_1 and x_2 while there does not exist a time-like linear combination of x_1 and x_2; $(x_1^*Fx_1)(x_2^*Fx_2) > |x_1^*Fx_2|^2$ if, and only if, the $n \times 2$ matrix X, whose column matrices x_1 and x_2 are linearly independent, is space-like.

These results may be extended to the case of p $n \times 1$ matrices x_1, ..., x_p where $3 \leqslant p \leqslant n$. The proof given above shows that the $p \times p$ matrix X^*FX, where X is the $n \times p$ matrix whose column matrices are x_1, ..., x_p, is singular if the p $n \times 1$ matrices x_1, ..., x_p are linearly dependent and so we shall suppose that they are linearly independent. The $p \times p$ matrix X^*FX cannot have less than $p - 1$ positive characteristic numbers (since every pair of linearly independent $n \times 1$ matrices possesses a space-like linear combination). Thus the $n \times p$ matrix X is space-like (by which we mean that every non-trivial linear combination of its column matrices is space-like) if, and only if, the determinant of the $p \times p$ matrix X^*FX is positive. This determinant is negative if, and only if, there exists a time-like linear combination of the p $n \times 1$ matrices x_1, ..., x_p. The matrix X^*FX is singular if, and only if, there exists a null non-trivial linear combination of x_1, ..., x_p (while no time-like linear combination of these exists). In the case $p = n$ the determinant of X^*FX is negative since there certainly exists a time-like linear combination of n linearly independent $n \times 1$ matrices x_1, ..., x_n; the negativeness of det X^*FX is *a priori* evident since det X^*FX is the product of det F by $|\det X|^2$.

If we shall ever have to consider physical spaces with more than one time direction we must introduce quasi-Lorentz spaces. Thus, for example, if we have two time-directions the $n \times n$ diagonal matrix G whose first $n - 2$ diagonal elements are 1, the last two being -1, takes the place of F for Lorentz spaces and of the $n \times n$ identity matrix E for Euclidean, or unitary, spaces. If we have q time directions G is the $n \times n$ diagonal matrix whose first $n - q$ diagonal elements are 1, the remaining q being -1; there is no lack of generality involved in taking $q \leqslant n/2$. An $n \times 1$ matrix x, real or complex, is space-like, null or time-like according as

x^*Gx is positive, zero or negative, respectively. If $x_1, \ldots, x_p$ are p linearly independent $n \times 1$ matrices the $n \times p$ matrix X is said to be space-like, null or time-like according as every non-trivial linear combination of $x_1, \ldots, x_p$ is space-like, null or time-like, respectively; thus X is time-like if, and only if, the $p \times p$ matrix X^*GX is positively definite; X is null if, and only if, X^*GX is the zero $p \times p$ matrix and X is time-like if, and only if, X^*GX is negatively definite. Every set of $q + 1$ linearly independent $n \times 1$ matrices contains a space-like linear combination and every set of $n - q + 1$ linearly independent $n \times 1$ matrices contains a time-like linear combination. If, then, $p > q$ the $p \times p$ matrix X^*GX cannot have less than $p - q$ positive characteristic numbers and, similarly, if $p > n - q$, X^*GX cannot have less than $p + q - n$ negative characteristic numbers. Thus, if $p = n$, X^*GX has exactly $n - q$ positive characteristic numbers and exactly q negative characteristic numbers. If $p = n - 1$ the sign of one of the characteristic numbers of X^*GX is uncertain (there being at least $n - q - 1$ positive characteristic numbers and at least $q - 1$ negative characteristic numbers). It follows that the necessary and sufficient condition that the space spanned by $n - 1$ linearly independent matrices $x_1, \ldots, x_{n-1}$ should have a q-dimensional time-like subspace is that the determinant of the $(n - 1) \times (n - 1)$ matrix X^*GX should have the sign of $(-1)^q$ and the necessary and sufficient condition that this space should have a $(n - q)$-dimensional space-like subspace is that this determinant should have the sign of $(-1)^{q-1}$. If $p = n - 2$, and $q \geqslant 2$, the signs of two of the characteristic numbers of X^*GX are uncertain and the necessary and sufficient condition that the space spanned by the $n - 2$ linearly independent $n \times 1$ matrices $x_1, \ldots, x_{n-2}$ should have a $(q - 1)$-dimensional time-like subspace and a $(n - q - 1)$-dimensional space-like subspace is that the determinant of X^*GX should have the sign of $(-1)^{q-1}$. If $q < p \leqslant n - q$ the matrix X^*GX has $p - q$ positive characteristic numbers and the signs of the remaining q characteristic numbers are uncertain. Thus, if $q = 2$, the necessary and sufficient condition that the space spanned by the p linearly independent $n \times 1$ matrices $x_1, \ldots, x_p$ should possess a $(p - 1)$-dimensional space-like subspace and that there should exist a time-like linear combination of $x_1, \ldots, x_p$ is the negativeness of the determinant of the $p \times p$ matrix X^*GX, $2 < p \leqslant n - 2$.

THE CHEMICAL NATURE OF THE ACROSOME
IN THE MALE GERM CELLS

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The close connection of the acrosome of the animal sperm with the Golgi apparatus was first suggested by Bowen[1] in 1923, who stated that the acrosomal material might be "secreted" from the Golgi apparatus. While his cytological studies seem to support the concept that the acrosome is derived from the Golgi apparatus, the staining methods employed do not allow any conclusions as to the chemical nature of either structure. The recent development of cytochemical procedures, using specific staining reactions for the chemical characterization of cellular constituents *in situ* now makes possible an analysis of some of the chemical components of the dictyosomal material[22] and the acrosome of the male germ cells. *Arvelius albopunctatus*, an hemipteran insect, provides an especially favorable material for the cytochemical studies, since the different lobes of the testis are characterized by a constant and marked difference in the size of their spermatocytes, spermatids and sperms.[2, 19, 20] In the present paper evidence is presented that the acrosome of the sperm is derived from the dictyosomes of the primary spermatocytes, and that both contain polysaccharides in a 1,2 glycol grouping. The amount of polysaccharides in the dictyosomal material and in the acrosome of spermatocytes, spermatids and sperms is strikingly higher in the large sized cells of the third and fifth lobes than in the normal and small sized cells of the remaining four lobes. For the identification of the polysaccharides in the cells of the testis we used the microchemical periodic acid Schiff(PAS) reaction resulting in the staining of polysaccharide structures in tissues as described by Hotchkiss[5] in 1948. According to Hotchkiss[5] and McManus,[12] the reaction of periodic acid with carbohydrates, when present as 1,2 glycol grouping, is considered to be the following:

$$\text{R}-\overset{\displaystyle |}{\underset{\displaystyle |}{\text{C}}}-\overset{\displaystyle |}{\underset{\displaystyle |}{\text{C}}}-\text{R} + \text{HIO}_4 \rightarrow 2\text{R}-\text{CHO}$$

$$\overset{\text{OH OH}}{}$$
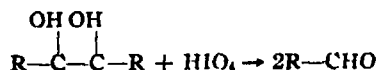
The aldehydes which are formed after periodic acid oxidation from 1,2 glycols in sections form a colored complex with the Schiff reagent. In order to characterize the type of polysaccharides in our tissues, we used the Hotchkiss reaction in combination with the acetylation technique of McManus and Cason[13] and with enzymes such as amylase, diastase and various hyaluronidases (derived from bull testis and from bacteria).[18]

Furthermore additional specific staining for desoxyribosenucleic acid (DNA) by means of methyl-green,[14] and for basic proteins by means of fast-green[20] was secured simultaneously in the same sections. The detailed technique of these procedures as well as a standardization of the Hotchkiss reaction for quantitative estimation of polysaccharides in tissues will be described.in another publication (in collaboration with Orbison and Lieb).

The testes of Arvelius[24] were fixed in Carnoy's acetic-alcohol and sectioned at 6, 10 and 16 microns. Staining was always performed in the same way under standardized conditions, and enzyme experiments, such as for instance the treatment with hyaluronidase, were made with sections directly adjacent to the control section (without periodic acid) and to the test slide (with periodic acid but without the enzyme). The amount of polysaccharides was judged on the basis of the intensity of the Schiff color after periodic acid oxidation in individual cells by the photometric microscopic method, as described by Schrader and Leuchtenberger[20] and with an apparatus of the type designed by Pollister and Moses.[15] Control sections, without periodic acid oxidation, did not show any development of color after exposure to the Schiff reagent. For the absorption measurements of the Schiff color in the sections, acrosomal material of spherical shape was selected in large and in normal sized cells which were in the same stage of development and were present in the same slide. Photometric measurements of the acrosome in the small sized cells were not possible, due to their small dimensions. The amounts of polysaccharides are expressed in arbitrary PAS units (periodic acid Schiff) and are obtained by multiplying the extinctions by the square of the radius of the sphere of the acrosome.

The third and fifth lobes of the testis, which contain the large sized cells, show the same picture in regard to size, as well as the PAS color of the dictyosomes and their behavior in the formation of the acrosome. The lobes carrying the normal and small sized cells show a markedly smaller amount of acrosomal material, though essentially the same steps of development can be observed.

The formation of the acrosomal material from the dictyosomes in the large sized cells, as seen in slides treated with PAS and counterstained with methyl-green may be outlined as follows:

(*a*) In the confused stage of the first spermatocytes, the dictyosomes tend to form larger aggregates in the vicinity of the nuclear membrane. This dictyosomal material shows the characteristic red stain resulting from the PAS treatment, a stain not shown by the nucleus, nucleolus and the cytoplasm.

(*b*) In the course of the two spermatocyte divisions, the dictyosomal material is distributed approximately equally to the resulting spermatids, and is present in the latter as a granular mass. This constitutes the so-

called acroblast. The large Nebenkern does not stain after PAS at this or any later stage.

(c) The acroblast gives rise to the acrosome which at first is applied to one side of the spherical nucleus as a round cap, staining an intense red after PAS treatment.

(d) In the succeeding stage, this cap appears to become more liquid and extends over half or more of the still spherical nucleus.

(e) When the nucleus elongates, the acrosomal material elongates simultaneously. But the long, pointed acrosome of the finished sperm is not molded solely by the lengthening of the sperm nucleus, since it extends far beyond the anterior tip of the latter.

While all the earlier stages (a–d) show the characteristic methyl-green staining of the DNA in the nucleus, no such color is discernible with certainty in the final stages. Apparently this is due to the fact that the disproportionately large amount of PAS positive material in the large cells completely covers the nucleus of the elongated phase. That DNA is present in such spermatids in a normal quantity has already been demonstrated by Schrader and Leuchtenberger.[20] Indeed, in the small and normal sized spermatids, in which the quantity of DNA is the same as in the large cells just described, there is no difficulty in observing the methyl-green stain of the DNA in the nucleus, for the relatively much smaller amount of acrosomal material does not obscure it.

TABLE I

COMPARISON OF AMOUNTS OF POLYSACCHARIDES (PERIODIC ACID SCHIFF REACTION) IN THE ACROSOME OF LARGE AND NORMAL SIZED SPERMATIDS OF ARVELIUS ALBOPUNCTATUS, BY MICROSCOPIC PHOTOMETRIC MEASUREMENTS

LOBE OF TESTIS	TYPE OF CELL	NUMBER MEASURED	ACROSOME MEAN DIAMETER IN MICRONS	MEAN EXTINCTION	POLYSACCHARIDES, MEAN AMOUNT IN ARBITRARY PAS UNITS PER ACROSOME
Third (large sized cells)	Early spermatid	10	4.75	1.0 ± 0.02	5.76 ± 0.16
Sixth (normal sized cells)	Early spermatid	10	0.76	1.0 ± 0.03	0.14 ± 0.04

From the cytological studies there seems to be no doubt that the PAS positive material of the acrosome in the spermatids and sperms is derived from the PAS positive material in the dictyosomes of the primary spermatocytes (confused stage). A similar "gradual transformation of the Golgi material of the young spermatid into the sperm cap and acrosome," also by the use of the Hotchkiss reaction, has been observed by Leblond[8] in the rat testis.

The striking increase of the amount of PAS positive material in the acrosome of the large sized cells as compared with the amount in the

normal sized cells is demonstrated in table 1. On the basis of the measurements tabulated in table 1, it appears that the acrosome of the large-sized spermatids contains considerably more polysaccharides (about 40 times more) than the acrosome of the normal sized spermatids in the same meiotic stage. This increase in carbohydrates in the large sized cells is in accordance with the previously reported increase in proteins and ribosenucleic acid of these cells[20] and supports the concept of Schrader and Leuchtenberger that in Arvelius the increase in volumes of the nucleus, nucleolus and cytoplasm in the large sized cells, as compared with those of the normal and small sized cells, represents a true growth.

TABLE 2

EFFECTS OF VARIOUS REAGENTS AND ENZYMES ON THE POLYSACCHARIDES (AS DEMONSTRATED BY THE PERIODIC ACID SCHIFF REACTION) OF THE DICTYOSOMAL AND ACROSOMAL MATERIAL OF THE GERM CELLS OF THE TESTIS OF ARVELIUS ALBOPUNCTATUS

REAGENT	CONCENTRATION	TIME OF EXPOSURE	TEMPERATURE	PAS REACTION
Acetic anhydride + Pyridine	13 cc. + 20 cc.	45 min.	Room	Negative
Acetic anhydride + Pyridine	13 cc. + 20 cc.	45 min.	Room	...
followed by KOH	0 1 N.	45 min.	Room	Positive
Methanol chloroform	1:1	24 hrs.	60°C.	Positive
Saliva	Conc.	30 min.	Room	Positive
Amylase (Fisher Scientific "Amylopsin"	1%	60 min.	37°C.	Positive
Diastase (Merck U. S. P. IX)	1%	60 min.	37°C.	Positive
Schering hyaluronidase bull testis A	4 T.R.U. per 1 cc.	24 hrs.	37°C.	Positive
Schering hyaluronidase bull testis B	3.3 T.R.U. per 1 cc.	24 hrs.	37°C.	Positive
Wyeth hyaluronidase bull testis	140 T.R.U. 70 T.R.U. per 1 cc.	24 hrs.	37°C.	Positive
Clostridium welchii hyaluronidase	.....	24 hrs.	37°C.	Positive

Some further analysis as to the chemical nature of this differential growth was attempted and from the results presented in the first two rows of table 2 it is evident that the chemical groups of the acrosomal material reacting with the Schiff reagent after periodic acid oxidation are aldehydes derived from 1,2 glycol grouping of carbohydrates. Using the reversible acetylation technique in tissue sections, as described by McManus and Cason,[13] the acetylation of the 1,2 glycols by acetic anhydride prevents the

formation of aldehydes after periodic acid oxidation and thus gives a negative PAS reaction, as seen in the first row of table 2. The removal of the acetyl groups from the acetylated 1,2 glycols by 0.1 N KOH restores the 1,2 glycol linkage and thus allows the formation of aldehydes after periodic acid oxidation, which color with the Schiff reagent, as seen in row 2 of table 2. That the positive PAS reaction is actually due to 1,2 glycols of carbohydrates and not to similar groups of glycolipids is shown by the experiments recorded in the third row of table 2; here the method devised by Gersh[4] showed that an extraction of glycolipids with hot methanol chloroform resulted in no effect on the positive PAS reaction. Furthermore it is obvious from table 2 that the polysaccharide content of the dictyosomal and acrosomal material is not due to the presence of starch or glycogen, because pretreatment of the cells with amylase, diastase or saliva did not change the positive PAS reaction. Control slides containing glycogen in liver cells, fixed and treated in the same manner as the testis slides of Arvelius, showed a negative PAS reaction of the glycogen granules after diastase and saliva treatment. It is further evident that different types of hyaluronidases, even in concentrations as high as 140 Turbidity Units (T.R.U.) per 1 cc., which readily digested the hyaluronic acid of umbilical cord, did not affect the PAS reaction of the acrosomal carbohydrates. These results more or less exclude the presence of hyaluronic acid in the acrosome and dictyosomal material of the germ cells of the Arvelius testis; although the possibility must be admitted that, due to a species specificity, the bull testis and bacterial hyaluronidases might not act on insect hyaluronic acid—or the substrate after fixation with Carnoy might be present in a form in which the enzyme is unable to attack it. It may be of interest to note that the treatment of the Arvelius testis slides with the enzyme solution of *Clostridium welchii* abolished the methylgreen stainability of the desoxyribose-nucleic acid in the nuclei of the cells and thus indicated the presence of a desoxydepolymerase in this enzyme solution. The cytochemical detection of a desoxydepolymerase in bacterial filtrates of *Clostridium welchii* is in good agreement with the observation of Warrack and coworkers,[21] who have obtained independently the same results by chemical means (personal communication).

Since the presence of polysaccharides with 1,2 glycol grouping in the dictyosomal material and in the acrosome of the male germ cells cannot be explained by its content of starch, glycogen or hyaluronic acid, the question arises as to the possible chemical nature of this substance. It is known that male germ cells of all species examined contain an enzyme hyaluronidase, which dissolves the cementing material surrounding the female germ cells and thus makes fertilization possible.[4, 11, 17, 18] Exceptions are the male germ cells of reptiles and birds, in which hyaluronidase has not been found and where accordingly the ova are not surrounded by

follicle cell cumuli.[6,7] Moreover, the acrosome of the sperm has already been regarded by early workers, such as Lillie[9] and Bowen,[1] as being of great importance for the fertilization process, especially in connection with the penetration of the sperm and the activation of the egg. Bowen,[1] who pointed out the close analogy between the formation of the acrosome and that of a "secretory granule," has suggested that in the case of the acrosome the Golgi apparatus may be a center for the formation of enzymes which may play a part in the activation of the egg. The existence of enzymes in the sperm and their possible importance for the process of fertilization has already been stressed by Lillie[9] and Loeb.[10] The presence and possible role of the enzyme hyaluronidase in the acrosome and its elaboration by the dictyosomal material of the spermatocytes therefore demands consideration. The studies of Riisfeldt[16] demonstrating that during rat spermatogenesis the hyaluronidase is first found in the spermatocytes may be a corollary to our findings of the appearance of the dictyosomal material in the primary spermatocytes and suggest a possible relationship between dictyosomal material and hyaluronidase. Whether the 1,2 glycol grouping of the polysaccharides in the dictyosomal material and the acrosome might be indicative of the presence of the enzyme hyaluronidase itself must await further chemical characterization of the enzyme Studies in our laboratory, in which bull testis hyaluronidase was tested in vitro for 1,2 glycol groups, gave a positive PAS reaction. Moreover, a series of preparations of this enzyme, containing respectively 220, 550, 900 and 1400 T.R.U. per mg., showed a corresponding increase in the intensity of the PAS reaction. Since, according to Hotchkiss, the amount of dye fixed is dependent upon the actual weight of glycol structure present, it seems that the more purified the enzyme preparation (for instance 1400 T.R.U. per mg. as compared with 220 T.R.U. per mg.), the more 1,2 glycol groups can be demonstrated by means of the PAS reaction. Whether the 1,2 glycol groups are actually a part of the chemical constitution of the enzyme hyaluronidase, or whether they happen to be an "impurity" which became more concentrated during the purification process of the enzyme, must await the testing of enzyme preparations with higher T.R.U. per mg., which are not yet available. The speculation that the enzyme hyaluronidase contains 1,2 glycol linkage and thus gives a positive PAS reaction if present in tissues, led us to investigate the snake sperm, in which, as previously mentioned, no hyaluronidase has been found, and to compare it with the bull sperm, which serves as a good source for the extraction of hyaluronidase. While both sperms show a distinct acrosome, the snake sperm showed only a very slight amount of PAS positive material at the extreme tip of the acrosome, in contrast to the bull sperm where the whole acrosome, which consists of a thin hull covering two-thirds of the sperm head, was stained by the PAS reaction. That the PAS positive reaction in

the acrosome of the bull sperm is not due to starch, glycogen or hyaluronic acid was shown by pretreating sections with amylase, diastase and bull testis hyaluronidases without any effect on the intensity of the PAS reaction.

Summary.—Evidence is presented that the acrosome of the sperm in Arvelius albopunctatus is derived from the dictyosomal material of the primary spermatocytes and that the dictyosomal material and the acrosome contain a polysaccharide with a 1,2 glycol grouping which is neither starch, glycogen nor hyaluronic acid. The amount of polysaccharides is approximately 40 times larger in the acrosome of the sperms derived from the large sized cells than in that derived from the normal sized cells. The possibility of the relationship between acrosome and the enzyme hyaluronidase is discussed.

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[22] We prefer to call the Golgi apparatus in the germ cells dictyosomal material since the dictyosomes have been seen frequently in living germ cells, while such structures as the Golgi network now appear to be fixation artifacts.

[23] We are indebted to Dr. J. Seifter of the Wyeth Institute of Applied Biochemistry for the generous supply of various samples of bull testis hyaluronidase; to the Department of Biochemistry, Schering Corporation, for bull testis hyaluronidase; and to Drs. Pillemer and Robbins of the Institute of Pathology, Western Reserve University, for a bacterial filtrate of *Clostridium welchii*.

[24] We are greatly indebted to Dr. S. Hughes-Schrader of Columbia University, who collected and fixed the Arvelius tissues for us in Costa Rica.

ON THE DERIVATION OF SPACE DENSITIES IN GLOBULAR CLUSTERS

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In studies of the distribution of stars in globular clusters, the observations give directly only the distribution of stars or integrated light as projected on a plane perpendicular to the line of sight. It remains to deduce the spatial distribution from this areal distribution. Early investigators of the structure of globular clusters gave their attention to this problem, and solutions were given by von Zeipel[1] and Plummer[2] for the case of a spherically symmetrical cluster.

Von Zeipel showed that the density (of stars or integrated light) in space at a distance ρ from the center of the cluster is given by

$$\varphi(\rho) = \frac{1}{\pi} \int_{\rho}^{R} \sqrt{r^2 - \rho^2} \, \frac{d}{dr} \left(\frac{1}{r} \frac{df}{dr} \right) dr, \qquad (1)$$

where $f(r)$ is the density in projection at a distance r from the projected center, and R is the radius of the cluster. (R may be infinite.) Plummer used a different approach, starting by dividing the cluster into a number of parallel strips of infinitesimal width dr. If $F(r)dr$ is the total number of stars (or the sum of integrated light) in a strip whose perpendicular distance from the projected center of the cluster is r, then the space density is given by

$$\varphi(r) = - \frac{1}{2\pi r} \frac{dF}{dr}. \qquad (2)$$

Plummer's formula is plainly the simpler of the two; and in the reduction of star counts, where $F(r)$ follows directly from the summation of the number of stars in successive reticle squares, it is to be preferred. In measures of integrated light intensity, however, the observed function is $f(r)$, and $F(r)$ can be derived only by numerical integration along the length of each strip. The reduction by this method is just as laborious as by direct application of von Zeipel's formula; in fact it is easy to show that the two procedures are equivalent. There seems in the case of integrated light intensities to be no way of getting around the labor of applying von Zeipel's formula.

Another drawback of von Zeipel's formula is the error introduced in carrying out the required differentiations and integration. The observational errors—or the errors of smoothing the raw data—are magnified at each successive step of the calculation; and the differentiation or quadrature

formulae have residual errors which are often considerable, because the observed points are not as closely spaced as the numerical analyst might desire.

It is possible, however, to improve the practical accuracy of von Zeipel's formula by eliminating one of the differentiations. Partial integration of the formula gives

$$\varphi(\rho) = -\frac{1}{\pi} \int_\rho^R \frac{1}{\sqrt{r^2 - \rho^2}} \frac{df}{dr} dr, \tag{3}$$

the integrated term vanishing. One differentiation has been eliminated, but the integral in this form is not amenable to straightforward numerical quadrature because of the divergence of the integrand at the lower limit.[3] Divergent integrands pose no such problem in analytic quadrature, however (provided the integral itself does not diverge); so we may remove the present difficulty by absorbing the divergent part of the integrand into the analytic quadrature leading to a new formula for numerical quadrature. In other words, we will write the integral as

$$\int_\rho^R \frac{1}{\sqrt{r + \rho}} \frac{df}{dr} \frac{dr}{\sqrt{r - \rho}} \tag{4}$$

and derive a new type of quadrature formula. Given a set of values of a function y, the new formula will give

$$\int y(r) \frac{dr}{\sqrt{r - \rho}} \tag{5}$$

(instead of the conventional $\int y(r)dr$) in terms of values of y.

Let $y_0, y_1, y_2, \ldots$ be the values of y at a number of equally spaced points $r_0 (=\rho), r_1, r_2, \ldots$. We define a variable u by

$$r = r_0 + uh, \tag{6}$$

where h is the spacing $r_n - r_{n-1}$. We need formulae only for the integral over the strip from r_0 to r_1, since elsewhere the conventional quadrature formulae are applicable. Substitution of u for r as variable of integration gives for the required integral

$$I = \int_{r_0}^{r_1} y \frac{dr}{\sqrt{r - r_0}} = \sqrt{h} \int_0^1 y \frac{du}{\sqrt{u}}. \tag{7}$$

We can now derive various quadrature formulae, depending on which points we choose to pass the approximating polynomial through.

Let us first approximate y by a straight line passing through the points (r_0, y_0) and (r_1, y_1). The Lagrange interpolation formula gives for the equation of the line

$$y = y_0 (1 - u) + y_1 u, \qquad \cdot \qquad (8)$$

and the approximation to the integral becomes

$$I = \sqrt{h} \int_0^1 [y_0(1 - u) + y_1 u] \frac{du}{\sqrt{u}} = \frac{2}{3} \sqrt{h} \, (2y_0 + y_1). \quad (9)$$

If we pass a quadratic through the points (r_{-1}, y_{-1}), (r_0, y_0), and (r_1, y_1), we get

$$y = y_{-1} \frac{u(u - 1)}{2} - y_0(u + 1)(u - 1) + y_1 \frac{u(u + 1)}{2} \quad (10)$$

$$I = \frac{2}{15} \sqrt{h} \, (- y_{-1} + 12y_0 + 4y_1). \qquad (11)$$

And for a quadratic passing through (r_0, y_0), (r_1, y_1), and (r_2, y_2),

$$y = y_0 \frac{(u - 1)(u - 2)}{2} - y_1 u(u - 2) + y_2 \frac{u(u - 1)}{2} \quad (12)$$

$$I = \frac{2}{15} \sqrt{h} \, (9y_0 + 7y_1 - y_2). \qquad (13)$$

Formulae giving a higher order of approximation may be derived in a similar manner. Note that (13) is generally less accurate than (11) and is to be used only where y_{-1} is unknown.

Von Zeipel's formula may thus be integrated numerically in the form (4). In the strip bounded by $r = \rho$ we use formula (9), (11), or (13), taking the function y as

$$y = \frac{1}{\sqrt{r + \rho}} \frac{df}{dr}. \qquad (14)$$

Elsewhere we apply the usual quadrature formulae to (3). This method of deriving the spatial density distribution in a spherical star cluster is quicker and more accurate than the conventional application of von Zeipel's formula.

I should like to thank Dr. Bart J. Bok for reading this manuscript and making helpful suggestions.

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[3] Von Zeipel gives (3) but passes over it as unsuitable for numerical evaluation.

THE THEORY OF MICRO-METEORITES. PART I. IN AN
ISOTHERMAL ATMOSPHERE*

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Introduction.—In 1946, during the great Giacobinid meteor shower,
H. E. Landsberg[1] collected several small magnetic particles that apparently
were associated with the shower. Since some of these particles, a few
microns in length, were extremely angular in shape (wedge-shaped and
opaque), it seemed unlikely that they could have been the end-products
of vaporizing meteors. Landsberg concluded that they must have been
stopped by the atmosphere *without being heated above their melting-points*.
As a result of his suggestion I have developed the present theory to in-
vestigate the process whereby temperature radiation can dissipate the
energy gained by encounters with atmospheric molecules sufficiently
rapidly to permit finite meteoric particles to be stopped without melting.
Some basic concepts of this theory have been discussed by E. Öpik[2] and an
application made in the case of an isothermal atmosphere.

The term *micro-meteorite* appears to be an appropriate designation for one
of these small particles.

In every sense the micro-meteorites represent the lower extreme to the
ascending sequence embracing *meteor, fireball* and *meteoritic crater formation*.
Hence, the theory is a limited meteor theory, partially applicable to the
unobservable beginning of a meteor.

We may now assume and later (Part II) prove that interaction between
the air molecules striking and those leaving the micro-meteorite may be
neglected. The molecular mean free paths, even after correction for the
relatively slow velocity of air molecules thermally emitted, are greater than
the linear dimensions of the micro-meteorite.

Let us suppose that the micro-meteorite presents a certain surface
area, A, to the atmosphere, which it encounters with velocity, V. This

surface may be an average frontal area in case the body is rotating, or a fixed area in case the body does not rotate. If $A(\phi, \theta)$, a function of the ordinary spherical coordinates, represents the actual cross-sectional profile of the micro-meteorite as seen from direction ϕ and θ, then the average frontal area, A_1, is given by

$$A_1 = \frac{1}{4\pi} \int_{\theta = 0}^{\pi} \int_{\phi = 0}^{2\pi} A(\phi, \theta) \sin \theta \, d\phi \, d\theta. \tag{1}$$

This average area, A_1, may or may not equal the frontal area A. The thermal radiating area, B, however, will be specific for a particle of a given shape; it is given by

$$B = 4A_1. \tag{2}$$

Let us assume that the temperature of the micro-meteorite is at all times uniform over the area B. This assumption is equivalent to an assumption that the heat conductivity is infinite or that the heat capacity is zero. We shall first develop the theory on the assumption that the heat capacity is negligible and later investigate the nature of the error made.

The air molecules impinge on the forward surface with relative velocity, V, because, by definition, the mean free path of the outgoing molecules relatively to the moving body is larger than its linear dimensions. To evaluate the energy transfer to the surface of the body we may make use of the concept of the accommodation coefficient, α, which is defined in terms of the kinetic energy of the air molecules, referred to the coordinate system of the moving body. The accommodation coefficient is, then, the actual loss of kinetic energy by the air molecules, as a result of the encounter, divided by the loss if all of them were momentarily to adhere to the surface and be re-emitted at the thermal velocity corresponding to the surface temperature. Since the thermal energies, both original and at the surface temperature, are relatively small compared to the kinetic energy at velocity, V, these energies may be neglected with an error less than 1% (minimum $V = 11.2$ km./sec.). Hence α represents the fraction of the molecular energy at velocity, V, that is transmitted to the micro-meteorite.

Since the air molecules will encounter the body with relative energies of the order of 8 to 800 electron volts, while the work function of the surface will be only a few volts, the molecules will certainly penetrate the surface for several molecular layers except at the lowest velocities. We must conclude that few of them will leave with high velocities; the losses by dissociation, excitation and ionization can be only a few volts. Hence α must be nearly unity at most velocities.

In air of density, ρ, the micro-meteorite will meet in time, dt, an air mass, dm_a, given by

$$dm_a = A\rho V \, dt. \tag{3}$$

The corresponding energy gain, dE_g, amounts to

$$dE_g = \frac{\alpha}{2} V^2 \, dm_a = \frac{\alpha}{2} A\rho V^3 \, dt. \tag{4}$$

Part of this energy will be utilized in raising the temperature of the meteoroid, part radiated by black-(or gray)-body radiation, part used in dissociation, excitation and ionization and perhaps part used in disengaging material from the surface. If the accommodation coefficient is defined to include the dissociation, excitation and ionization and if vaporization is negligible, we may deal here explicitly with only the heating and radiation terms.

We may assume that the meteoroid was previously in temperature equilibrium with the night side of the Earth (or Sun and Earth during the day) at temperature T_0. With a gray-body emissivity coefficient of β, the loss of energy by radiation, dE_r, of a surface at temperature T_s is

$$dE_r = \beta B\sigma \, (T_s^4 - T_0^4)dt, \tag{5}$$

where σ is the Stefan-Boltzmann constant.

If the meteoroid is small ($s \ll 1$ cm.), of mass, m, and if the coefficient of heat conductivity is at all comparable to that of ordinary rocks, the internal temperature should differ negligibly from the surface temperature in time intervals somewhat smaller than one second. The permissible limits to this assumption will be discussed later. If, then, the heat capacity per gram is C_s, the temperature will vary as

$$mC_s dT_s = dE_g - dE_r. \tag{6}$$

By equations (4) and (5), equation (6) becomes

$$mC_s \frac{dT_s}{dt} = \frac{\alpha}{2} A\rho V^3 - \beta B\sigma(T_s^4 - T_0^4). \tag{7}$$

The precise conditions under which the heat capacity in the left member of equation (7) can be neglected are not apparent *a priori*. We can, however, easily determine a resultant rough limit to the dimensions of the micro-meteorite and later study the question more thoroughly. The maximum temperature, T_m, to which a micro-meteorite can be heated without appreciable vaporization is just below the melting point of the least refractory material in the meteoroid, approximately 1200°K. to 1700°K. for typical stones.[3] Iron, iron oxides and silica also fall within this range. The temperature rise from the equilibrium temperature at the Earth to T_m is relatively large. Generally this rise will occur over a considerable distance through the atmosphere since the radiation varies as T_s^4 while the

heating is proportional to atmospheric density. We will find that the heat capacity is negligible for fast micro-meteorites because they are so small. For slow micro-meteorites an atmospheric density increase of a factor of two will require roughly a half-second of atmospheric traverse at normal incidence.

Hence, if the heat radiation in an interval of the order of half-second near maximum temperature is large compared to the heat required to raise the temperature from T_0 to T_m we may safely neglect the heat capacity in equation (7). The condition just defined is

$$2mC_i\,(T_m - T_0) \ll \beta B\sigma\,(T_m{}^4 - T_0{}^4). \tag{8}$$

The limiting radius, s, for a spherical meteor of density, ρ_s, is, from equation (8)

$$s \ll \frac{3\beta\sigma}{2C_i\rho_s}\,(T_m{}^3 + T_m{}^2T_0 + T_mT_0{}^2 + T_0{}^3). \tag{9}$$

The right member of equation (9) is of the order of 0.01 cm. for an iron or stony meteorite. Hence we may safely ignore the heat capacity of micro-meteorites of radii less than 10 microns.

With our current assumptions then, we may set the left-hand member of equation (7) equal to zero and determine the surface temperature of a micro-meteorite as a function of its velocity and the atmospheric density. The result is

$$T_s{}^4 - T_0{}^4 = \frac{\alpha A\rho V^2}{2\beta B\sigma}. \tag{10}$$

While the temperature of the micro-meteorite is rising, its velocity is being reduced by atmospheric resistance. We may define the drag coefficient, D, by the equation

$$mdV = -\frac{AD\rho V^2}{2}dt. \tag{11}$$

The drag coefficient will include a major component, of the order of 2 numerically, if we assume that air particles momentarily adhere to the meteoroid and are reemitted isotropically. A smaller additional term will arise from the non-isotropic reemission according to the hypothesis of Tsien[4] and Miss Heineman.[5] No term will arise from evaporation of material, however, since we assume (perhaps erroneously at high velocities) that evaporation is negligible.

A rigorous inclusion of the reemission drag term would involve the surface temperature of the micro-meteorite. Since the effect is rather small, we may include it approximately after a solution has been made with D constant.

The simultaneous solution of equations (10) and (11) will provide a relation among the temperature, velocity and time, if the atmospheric density can be expressed as a function of the time. A complicated, variable and poorly known relation between atmospheric density and height actually exists at the atmospheric heights concerned. In the following sections the fundamental equations will be solved for certain types of such relations.

The Solution in an Isothermal Atmosphere.—Let us assume that the atmosphere is isothermal and that the mean molecular weight of the air is also constant. Then the atmospheric density, ρ, varies with the height, h, above sea level according to the relation

$$\rho = \rho_0 e^{-bh}, \tag{12}$$

where b, the logarithmic density gradient, is a positive constant and ρ_0 is constant.

Let us neglect the curvature of the Earth. Then for a particle entering the atmosphere with velocity, V, from an apparent radiant at zenith distance, Z, the time and height are related by the equation

$$dh = -V \cos Z \, dt. \tag{13}$$

Since meteoric velocities are so large we may neglect the effect of the Earth's gravity, assuming that the pertinent part of the trajectory is a straight line, and that the velocity is unaffected by gravity.[6]

Equations (11), (12) and (13) then lead to the following differential relation between velocity and height:

$$\frac{dV}{V} = \frac{A D \rho_0}{2m \cos Z} e^{-bh} \, dh. \tag{14}$$

We have already assumed that all of the quantities in equation (14) except V and h may be treated as constants. Hence we may integrate equation (14) between the limits of V_∞, the initial velocity at great heights essentially infinity, to any pertinent velocity, V, and height, h. The integral of equation (14), when combined with equation (12) to eliminate the height explicitly, yields the following relation between the velocity and atmospheric density:

$$\log (V/V_\infty) = -\frac{A D \rho}{2bm \cos Z}. \tag{15}$$

Now the micro-meteorite can traverse the atmosphere undamaged only if the velocity is reduced sufficiently to prevent the surface temperature from exceeding a critical temperature, T_m, somewhat below the melting point of the meteoritic materials. This restriction can be imposed if we eliminate the density, ρ, between Equations (15) and (10) and then impose a maximal condition on T_s. The first step gives

$$T_e{}^4 - T_0{}^4 = -\frac{abm \cos Z}{\beta\sigma BD} V^3 \log (V/V_\infty). \qquad (16)$$

To determine the maximum temperature from equation (16) we must make assumptions as to the functional forms of the accommodation coefficient, α, and the emissivity factor, β. In view of our lack of knowledge of the detailed physical and chemical structure of the micro-meteorites and, even with this knowledge, the uncertainty in the physical laws for α and β, we may as well adopt constant values for these quantities as well as for D. Hence, from the derivative of equation (16) the maximum temperature is reached at the critical velocity, V_c, given by

$$\log (V_c/V_\infty) = -\frac{1}{3}, \qquad (17a)$$

or

$$V_c = V_\infty/e^{1/3} = 0.7165 \ V_\infty, \qquad (17b)$$

where e is the base of the natural logarithms.

We may now solve for the ratio of the mass to the effective surface area, m/B, of the micro-meteorite from equation (16) for the maximum temperature, T_m. The resulting critical ratio is

$$m/B = \frac{3e\beta\sigma D(T_m{}^4 - T_0{}^4)}{ab \cos Z V_\infty{}^3}. \qquad (18a)$$

Equation (18a) represents the maximum value of m/B for a micro-meteorite that is to be stopped, undamaged by the atmosphere. The maximum radius for a spherical particle of radius, s (sphere) and density, ρ_s, is then

$$s \text{ (sphere)} = \frac{9e\beta\sigma D(T_m{}^4 - T_0{}^4)}{\alpha\rho_s b \cos Z V_\infty{}^3}, \qquad (18b)$$

a result corresponding closely to Öpik's Equation (53).[2]

It is of interest to note from equations (18) that the maximum particle dimension depends directly upon the critical temperature to the fourth power and upon the inverse cube of the original velocity. Low velocity and high melting point strongly favor the passage of such a particle through the atmosphere. Irregular or elongated shape favors the process for a given meteoric mass. Because of the $\cos Z$ term, larger masses may enter at lower angles of incidence.

For a rapidly spinning micro-meteorite in the shape of a right circular cylinder of length l and radius s (cyl.), the maximum radius in terms of the comparable sphere (equation 18b) of the same density and m/B ratio is given by

$$s \text{ (cyl.)} = s \text{ (sphere)} \times \frac{2}{3}\left(\frac{s+l}{l}\right) \text{ (cyl.)}. \tag{18c}$$

If the rapidly rotating cylindrical meteorite is to have the same radius as the spherical one, then the length must be equal to the diameter of the sphere. An extremely long cylindrical meteorite ($l \gg s$) will be stopped when its radius is 2/3 or less the corresponding spherical radius (equation 18b). The maximum permissible dimensions of micro-meteorites with other shapes·or orientations can be determined by means of equations (1), (2) and (18).

In applying equations (18) approximately to an atmosphere of variable temperature it is necessary to obtain an appropriate value of the logarithmic density gradient, b. The critical altitude is at the point of maximum temperature for the micro-meteorite. From equations (15) and (17) we find the critical density, ρ_m, at maximum temperature T_m as follows:

$$\rho_m = \frac{2bm \cos Z}{3AD}. \tag{19}$$

If the frontal area of the meteorite, A, is approximated by the average area, A_1, equations (2) and (18a) transform equation (19) into

$$\rho_m = \frac{8e\beta\sigma(T_m{}^4 - T_0{}^4)}{\alpha V_\infty{}^3}. \tag{20}$$

The value of b and the height corresponding to ρ_m may be derived from some standard atmosphere. The fact that the shape and mass of the micro-meteorite and $\cos Z$ do not enter equation (20) is rather surprising. In case there is reason to believe that the actual frontal area differs from the average cross-sectional area, a correction term can easily be applied for this factor in equation (20).

The drag coefficient, D, is a vital factor in equations (18) and (19). In case the air molecules impinging on the surface of the micro-meteorite are momentarily "captured" by the surface, but very quickly reemitted with energies corresponding to the temperature of the surface, D is equal to 2 with a small correction term for the Tsien-Heineman effect mentioned above. This situation corresponds to that when the accommodation coefficient, α, is exactly unity. M. L. Wiedman[7] finds that the accommodation coefficient for air on various metals is generally close to 0.9. At the higher energies here considered, there is little doubt that the air molecules will largely penetrate the surface molecular layers and be momentarily captured. Since only a few can combine chemically and since condensation is impossible at the temperatures considered, there appears to be little question that essentially all of the air molecules will be captured and then reemitted with an average velocity, v_T, corresponding to the temperature of the surface. The average velocity is given by

$$(v_T)^2 = \frac{8k_1 T_s}{\pi \mu_1},\tag{21}$$

where k_1 is the gas constant and μ_1 is the molecular weight of the gas.

If the micro-meteorite is assumed to be spherical and the reemission is uniform with respect to solid angle from a small area of the surface, the momentum transfer to a hemisphere per average molecule is $4\mu v_T/9$. Since the time lag of reemission must be small compared to a possible rotation period of the micro-meteorite, this momentum transfer will oppose the motion of the body. If we further accept $D = 2$ as the best approximation to the drag coefficient without including reemission, the value of D becomes:

$$D = 2[1 + 4v_T/(9V)].\tag{22}$$

A numerical calculation shows that the v_T-correction in equation (22) amounts at most to a few per cent in the velocity and temperature ranges under consideration. Most of the deceleration of interest occurs fairly near the critical region of maximum temperature. In adopting a constant value of D, therefore, we may use v_T applying at the maximum temperature and compensate this error somewhat by adopting $V = V_\infty$ in equation (22).

It would be desirable to make some correction in the drag coefficient for the fact that some of the impinging air molecules must deviate from the assumed process of penetration and thermal reemission, but we have no detailed information as to the surface characteristics of micro-meteorites. The relative error made in D by this omission is less than $1 - \alpha$.

The remaining undetermined constant in Equation (18) is the emissivity factor, β. Nothing precise can be known about this factor until micro-meteorites have been studied carefully in the laboratory. Probably β is very near unity. For want of better information we may set $\alpha = \beta \cong 1$, so that their effects cancel in equation (18). We may adopt $T_m = 1600°K.$, and $T_0 = 300°K.$ The remaining quantities to be specified in equation (18a) concern the atmosphere. The Tentative Standard Atmosphere of the National Advisory Committee for Aeronautics[6] represents good modern estimates of upper-atmospheric densities and will be used for the present calculation.

For a velocity of 23 km./sec., corresponding to the 1947 Giacobinid Meteor Shower, the atmospheric density at which a micro-meteorite of limiting dimensions attains maximum temperature ($\rho = 8.2 \times 10^{-10}$ gm./cm.^{3}) occurs near the 112-km. level, by application of equation (20). With an air temperature of 346°K. and $b = 1.1 \times 10^{-6}$ cm.$^{-1}$ the maximum radius of spherical iron particles for $\cos Z = 0.45$ are then calculated to be about 4 microns. Long cylindrical particles might have a diameter as great as 6 microns, in good agreement with Landsberg's observations mentioned earlier.

Part II of this paper will concern the solution of limiting dimensions for micro-meteorites in an atmosphere of constant temperature gradient and in the general case. A critical discussion of the assumptions adopted here will be included as well as some elaboration of the numerical results.

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MERCAPTAN-INDUCED COAGULATION OF PROTEINS*

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In this paper it will be demonstrated that mercaptans possess the property of inducing coagulation of certain proteins at room temperature and neutrality. This effect throws light on the importance of —S—S— bonds in the intramolecular folding of several proteins in the native state.

While the dispersive action of mercaptans on keratin is well known, the coagulative action on soluble proteins apparently has not been described. Goddard and Michaelis[1] found that wool readily dissolves at room temperature in thioglycollate solutions at pH 10–13 because of reduction of disulfide bonds. The following reaction[1-4] occurs in the thiol-disulfide system:

$$RS—SR + 2R'SH \rightleftharpoons 2RSH + R'S—SR'$$

Since greater alkalinity was required in the experiments of Goddard and Michaelis than was needed for the reduction of cystine by thiols, they[1] postulated that an additional intramolecular bridge must be broken before disulfides of keratin can be reduced; they assumed that the second bridge was salt-like in character and that it was disrupted by removal of a proton from the amino group in alkaline solution. Jones and Mecham[4] observed that a variety of keratins were dispersed at pH 7 and 40°C., by adding urea or anionic detergents to the mercaptan solution. Mirsky and Anson[5] found that thioglycollate in excess completely reduces disulfide groups in

denatured plasma albumin and egg albumin; in contrast sodium sulfite reduces only one-half of the cystine present.

Method.—Crystalline bovine plasma albumin and egg albumin and refined bovine γ-globulin (95 per cent homogeneous on electrophoresis) were studied. Acetylated bovine albumin was prepared by the method of Hughes as described by Olcott and Fraenkel-Conrat.[6] Analysis indicated that 78 per cent of the free amino groups were acetylated.

Unless otherwise stated the proteins were dissolved in 0.1 M phosphate buffer, pH 7.4, and the experiments were carried out in 13 × 100 mm.
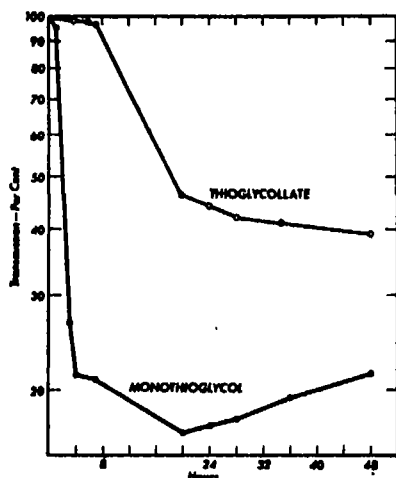
FIGURE 1

Rates of development of turbidity in plasma albumin induced by 0.2 M solutions of monothioglycol, and sodium thioglycollate. In each case the system consisted of bovine plasma albumin 50 mg., mercaptan compound 2 m. eq. and 0.1 M phosphate buffer, pH 7.4, to make the final volume 10 ml.; temperature 37°.
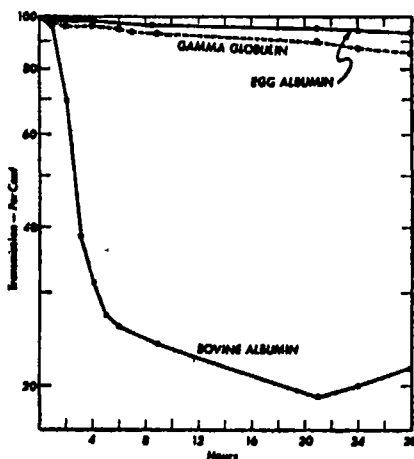
FIGURE 2

Differential rates of development of turbidity in various refined protein fractions induced by 0.2 M mercaptoethanol. The system consisted in each case of 50 mg. protein, mercaptoethanol 2 m. eq., and 0.1 M phosphate buffer pH 7.4 to make the final volume 10 ml.; temperature 37°.

glass tubes at 37°. Turbidity was determined in a Coleman spectrophotometer at λ = 660 millimicrons, using 19- × 150-mm. tubes. A protein solution was considered to be coagulated[7] when it failed to pass through a clean dry stainless steel screen (8 mesh, wire diameter 0.7 mm.).

Experimental.—When a mercaptan is added to a solution of bovine plasma albumin turbidity soon develops and increases to a maximum (Fig. 1), then decreasing slightly. The viscosity of the solution increases until a firm opaque gel forms. Egg albumin and γ-globulin behave in a similar fashion except that the reaction is much slower (Fig. 2). Dialysis

for 5 days against water produces no apparent change in these white mercaptan-induced gels.

An inverse relationship exists between the protein concentration and the minimal quantity of thioglycollate required to form a gel; at pH 7.4 and 37° the thioglycollate requirement decreased progressively from a concentration of 0.34 M to 0.08 M as the albumin concentration was increased from 1.2 to 5 per cent. At 5°, however, neither turbidity nor gels developed in 5 per cent albumin even after 72 hr. in 0.4 M thioglycollate.

In solutions of 7 per cent albumin, turbidity with subsequent gelation developed with the following agents: mercaptoethanol (monothioglycol), sodium thioglycollate, reduced glutathione, cysteine and sodium hydrosulfite. Mercaptoethanol produced turbidity more rapidly (Fig. 1) and gel formation at a lower concentration than did the other reducing agents employed. No gels or turbidity occurred in 7 per cent albumin solutions with the following reagents in concentration up to 0.4 M: sodium cyanide, potassium ferrocyanide, hydroquinone or sodium sulfite. Slight turbidity but no gel formation occurred in 0.4 M sodium ascorbate.

Coagulation of albumin by thiol compounds is favored by an increase in pH of the mixture. With increasing pH the minimum concentration of sodium thioglycollate required to form a gel in 2.5 per cent solutions of plasma albumin decreased progressively from 0.18 M at pH 7.0 to 0.06 M at pH 8.4. No monophasic gels were observed in albumin solutions treated with thioglycollic acid at pH values below 6.8. Between pH 5 and 6.6 the protein solution remained clear in 0.2 M thioglycollic acid while at pH 4.5 a synerizing precipitate formed.

Variation of the salt concentration over the range 0.1 to 1.1 M had no apparent effect on mercaptan-induced gelation of albumin. At salt concentrations less than 0.02 M gel formation was markedly inhibited, while at salt concentrations of 2–3 M the coagula formed were quite friable. Oxygen apparently plays no part in the mercaptan-induced coagulation of albumin; solutions degassed by repeated freezing and thawing *in vacuo* and sealed in the limbs of Λ-tubes evacuated to 10^{-3} mm. of Hg before mixing behaved identically with control mixtures exposed to air.

Partial acetylation (78 per cent) of the amino groups of plasma albumin resulted in a twofold increase in the mercaptan concentration required to form a gel; in a 2.5 per cent protein solution at pH 7.4, 0.27 M thioglycollate was required instead of 0.15 M as with unmodified albumin. The gels thus formed from acetylated albumin were markedly less turbid than those formed from unmodified albumin itself.

The opaque gels formed by the mercaptans are rapidly dissolved by the addition of urea in final concentration of 5 M or greater with nearly complete disappearance of the turbidity. Now urea itself possesses the ability to induce gelation of albumins and certain other proteins.[5] [6] These

coagula are quite different from mercaptan induced gels, being translucent and very cohesive. The protein gels induced by urea are dissolved rapidly by the addition of mercaptans.[10]

Discussion.—Two types of chemical bonds have been established as forces which maintain the protein molecules in specific configuration, namely, hydrogen bonds and disulfide bridges. In the theory of Mirsky and Pauling[11] the polypeptide chain of a native protein is folded and retained in its unique configuration by hydrogen bonding between the peptide nitrogen and oxygen atoms and also between the free amino and carboxyl groups of the diamino and dicarboxylic amino acid residues. Other workers have postulated that cystine[12] must form one of the side links between adjacent polypeptide chains in the protein molecule.

Classes of compounds which disrupt either type of linkage lead to the denaturation of a protein such as plasma albumin. Amides are well-known hydrogen-bond-forming substances which can rupture the hydrogen bonds involved in maintaining the protein in the native state; mercaptans reduce —S—S— linkages. With each type of reagent the denatured molecules aggregate, the gels varying in properties because of differences in the denatured protein molecule.

The coagulation of albumin by urea has recently been interpreted[10] as follows: Concentrated amides by competition with intramolecular hydrogen bonds of the native protein disrupt its structure and make disulfide groups reactive to small concentrations of sulfhydryl. By means of a chain reaction between protein —SH and —S—S— groups a reticulum is then knitted together consisting of intermolecular protein disulfide bonds which hold together the unfolded protein chains. Solvent is bound to the protein within this framework so that clear firm gels result.

Mercaptans appear to coagulate certain soluble proteins by a different mechanism, namely, disruption of the protein configuration through the cleavage of disulfide bonds followed by aggregation of the denatured protein through hydrogen bonds. Plasma albumin undergoes these changes more rapidly than γ-globulin or egg albumin implying that the disulfide groups of albumin are more readily available to reduction of —SH groups than in the other proteins.

The ready dispersion of the opaque mercaptan-induced gels upon the addition of urea indicates that aggregation in these gels is chiefly through intermolecular hydrogen bonding. Further evidence is derived from the deficient capacity of acetylated albumin to coagulate in the presence of mercaptans, due to a decrease in the number of potential hydrogen bond formers, the amino groups. The absence of any oxygen effect in mercaptan-induced gelation makes it doubtful that reoxidation of reduced disulfide bonds plays a major part in the aggregation.

Summary.—Mercaptans and hydrosulfite denature and subsequently

coagulate bovine plasma albumin, γ-globulin and egg albumin to form opaque gels at room temperature and neutrality. The reaction with bovine plasma albumin is many times more rapid than with the other two proteins. Gels so formed rapidly dissolve in concentrated solutions of amides such as urea. The capacity of albumin to form this type of gel is decreased by partial acetylation of the albumin. The evidence indicates that mercaptan-induced gelation occurs through hydrogen bonding between the extended denatured protein molecules.

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THE ACTION SPECTRUM OF THE INHIBITION OF GALACTOZYMASE PRODUCTION BY ULTRAVIOLET LIGHT*

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Certain yeasts, when placed in contact with galactose, acquire the ability to use that substrate after a period of time. Such a response is called enzymatic adaptation and will take place even in the absence of a nitrogen source. In recent years, with the realization that at least one of the components of any enzyme is a protein, it has been recognized that in adaptive enzyme formation we have a case where specific protein material is synthesized by resting cells.

It has been demonstrated that ultra-violet radiations from a sterilamp inhibit the aerobic adaptation of yeast to galactose.[1] Determination of an action spectrum for this effect might enable us to identify the cellular constituent affected by the radiation. The problem is of theoretical in-

terest because a clue might be found to the mechanism of adaptive enzyme synthesis. An action spectrum has been determined and is reported herein.

The theoretical basis and the limitations of the action spectrum method are discussed by Loofbourow.[2] The reciprocals of the energy required to produce a given effect when plotted against the wave-length give a curve which is called the action spectrum. This is thought to correspond to the absorption spectrum of some photolabile compound responsible for the biological effect being considered; the absorption spectra of known compounds are matched with the action spectrum. A summary of ultraviolet action spectra is given by Giese.[3]

Experimental.—In the present experiments the same strain of yeast was used as in the previous experiments.[1] Cultures were grown at 28°C. for 24 hours in liquid medium (30 ml. 3% dextrose, 1% yeast extract in 250 ml. flasks) on a mechanical shaker. The cells, washed as before, were suspended for experiments in M/20 KH$_2$PO$_4$ in a concentration of 2 × 10^8 cells per ml.

TABLE 1

MINIMAL DOSES REQUIRED TO PREVENT GALACTOZYMASE FORMATION FOR AT LEAST FOUR HOURS

WAVE-LENGTH, Å.	DOSE, ERGS/MM.[2]
2345	3374
2537	1687
2654	1687
2804	3374
3025	13,596
3130	>54,384

Ultra-violet light obtained from a quartz mercury arc was passed through a quartz monochromator, and the intensity of the light was measured with a thermopile galvanometer system as described elsewhere.[4] The monochromatic light struck the suspension of cells from below. The volume of cells irradiated was 0.06 ml. Stirring during irradiation was performed by a moving jet of compressed air saturated with water vapor. Precautions were taken against evaporation; if evaporation was excessive, the sample was discarded. Once irradiation had begun, the sample was handled only in red or yellow light to prevent photoreactivation.

Following irradiation, the sample was transferred to the reaction cup of a capillary microrespirometer of a modified Kirk-Stern type,[5] developed by the author and Mr. Robert Perthel of the Stanford Research Institute. A 0.06-ml. sample of 4% galactose solution was added to the suspension with a braking pipette, and the course of the respiration followed by recording the progress of a kerosene drop in the capillary tube. Thermal regulation of the system was accomplished by submerging the cups at either end of the U-shaped capillary in a vessel of water at room tempera-

ture.   Stirring the yeast in the reaction cup to keep it well aerated was performed by means of a tiny magnetic flea made of a piece of fine steel wire sealed in a glass capillary.

The inhibiting effects of six different wave-lengths were studied: 2345, 2654, 2804, 3025 and 3130 Å.   For comparison of efficiency, determinations were made of the minimum doses required to prevent adaptation for four hours after addition of galactose.   Normal adaptation time of this strain of yeast is 90 minutes.[1]   The criterion of adaptation was a measurable increase in the rate of respiration above the endogenous before 240 minutes had elapsed.   In doubtful cases the experiment was followed for another hour.   Table 1 shows the results which were obtained, each being the result of at least three series of experiments.
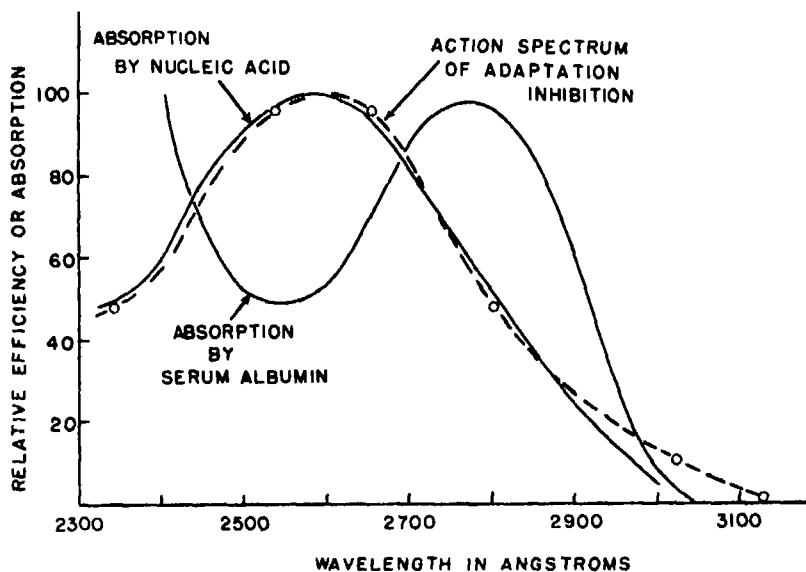
FIGURE 1

Action spectrum for inhibition by ultra-violet light of adaptation of yeast to galactose.

Analysis of the above data reveals that there is maximum efficiency in the region of 2600 Å. with a decrease on either side.   If the relative efficiency is plotted against wave-length so that the maximum of the action spectrum is at the same height as that of the absorption spectrum of nucleic acid, it is seen that there is excellent agreement between the two.

Discussion.—We have seen in Fig. 1 that the absorption spectra of unconjugated proteins such as serum albumin differ markedly from that of nucleic acid.   Unconjugated proteins, due to the aromatic amino acids they contain, have an absorption peak in the region of 2800 Å.; nucleic acids absorb maximally at 2600 Å. because of their purine and pyrimidine

rings. On the basis of present evidence and theory, it would have been reasonable to expect that we would obtain an action spectrum of either, since both have been implicated in protein synthesis. The subject of synthesis of proteins, including adaptive enzymes has been reviewed by Northrop.[6] He points out that enzymes, themselves proteins, must catalyze the formation of other proteins. In addition, Northrop advances the hypothesis that protein synthesis occurs in two steps. The first is the auto-catalytic formation of proteinogens or "type" proteins. The second step is the production of stable proteins by either catalytic or non-catalytic reactions. The first requires energy, the second does not. The proteinogen, though hypothetical, is thought to contain nucleic acid because of its autocatalytic formation. Spiegelman also assigns a prominent role to nucleoprotein in his plasmagene theory of adaptive enzyme formation.[7] Spiegelman and Kamen correlated the phosphate turnover of the fraction of yeast containing nucleic acid with adaptation.[8] They stated later however, that their results might apply equally well to constituents in the fraction.[9]

Although it is difficult at this time to say where nucleic acid acts in the production of galactozymase, it is evident from the results presented in this paper that it is of considerable importance. If we allow ourselves to generalize, it would seem that all other protein synthesis would be adversely affected by ultra-violet light. This is not hard to imagine, considering the lethal effect of ultra-violet radiations; death is the logical fate of an organism which cannot replace its outworn protoplasm. It is significant in this regard that the action spectrum for suppression of division of yeast and bacteria also resembles absorption by nucleoprotein.[3, 10, 11]

Summary.—The action spectrum of ultra-violet light inhibition of adaptation of yeast to galactose was determined. The results strongly indicate that nucleic acid is the cellular constituent affected by the light. It follows that nucleic acid is at least partially responsible for the adaptive enzyme production. The possible significance of these findings is briefly discussed.

* Part of a thesis submitted as a partial requirement for the Ph.D. The author is indebted to Dr. Arthur C. Giese who suggested the problem and helped in its execution.

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THE STRUCTURE OF THE HETEROCHROMATIC PART OF THE Y-CHROMOSOME IN DROSOPHILA BUSCKI*

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In the salivary glands of *D. buscki* the Y-chromosome was at first shown to be a small euchromatic element whose length is about ¹/₃₄ of the X-chromosome, and consisting of some 14–15 bands.[1,2] In ganglial metaphases, this Y-chromosome has the form of a tapering rod, narrow at the proximal end which carries a small knob-like thickening; the length is about equal to the X-chromosome. This non-correspondence between the appearance of the Y-chromosome in the two types of cells was attributed to a heterochromatic or inert part, which is greatly reduced in relation to length, or apparently absent, in salivary glands. Until recently few attempts were made to identify such heterochromatic elements in salivary gland cells.

It has been shown previously that the Y-chromosome in *D. buscki* is necessary for the life of males: XO-males die at an early stage of the development.[3-5] As a result of a comprehensive cytogenetic investigation on the Y-chromosome in *D. buscki*, it has been possible to clarify the role of constituent parts in some detail, and to show their action on the development of males. In particular, it has been shown that the Y-chromosome is two-armed: the right, short arm, is the knob-like thickening at the proximal end, and is the euchromatic element in the salivaries; the left, long arm, is the inert heterochromatic part (unpublished).

New information concerning the structure of this inert part has also been obtained in the course of this work, and is reported in the present paper.

Experimental Data.—The material for this work was the mutant line *Curled-Y(404)* obtained in an F_1 from crossing X-rayed males to wild type females. The ends of the wings of the mutant flies are bent upwards. This mutant behaves genetically as Y-linked, that is, it is inherited from father to son. Among the offspring of mutant males, about 10–12% of non-mutant males appear. This last class of males have a slightly shortened body, and are completely sterile.

Cytological analysis of the mutant males shows a break in region 7 of IIR. The proximal part of the broken IIR usually pairs fully with its

unbroken partner, while the distal part usually pairs only partially, or lies entirely free in the nucleus near the proximal ends of the other chromosomes. In the class of sterile males mentioned above (second class) a trivalent for the distal part of IIR is seen. This third, short element, which is the distal part of IIR seen in mutant males, sometimes pairs with its normal partners, or sometimes is free. The euchromatic Y-chromosome element in either type of male is not involved in the abberation. In ganglial metaphases of mutant males there is one autosome with a markedly longer arm and a Y-chromosome which is short and two-armed. The males of the second class have normal autosomes, but also a short two-armed Y-chromosome (these males are hypoploid for a part of the Y and hyperploid for the distal part of IIR).
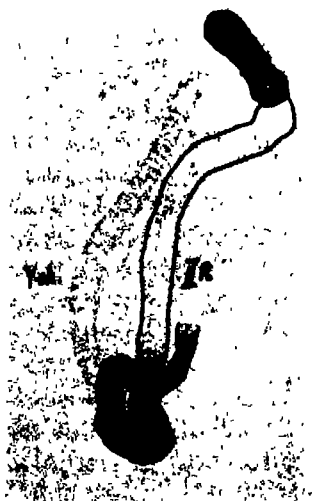
FIGURE 1

Translocation involving the left arm of the Y and the right arm (IIR) of the second chromosome in *D. buscki*.

From all these data, it is apparent that we are here dealing with a reciprocal translocation between the Y and IIR. The position of the break in IIR is known; in the Y-chromosome, the break is in the proximal part, near its knob-like end. One arm of the new Y-chromosome is this knob-like thickening, the other arm is the distal part of the translocation IIR. The long heterochromatic arm of the Y-chromosome is translocated to the top of the proximal part of IIR, and the lengthened arm thus formed can be seen in an autosome in metaphase preparations. This last circumstance has given us the opportunity to observe in salivary gland preparation the translocated heterochromatic part of the Y-chromosome. It can be seen at the end of the proximal part of IIR. If therefore, this heterochromatic part is represented by some sort of structure, this structure must be located at the end of the proximal part of IIR.

At first, all attempts to find any such structure were unsuccessful. However, trials of culture media were made to secure better development of *D. buscki* larvae, and this was responsible for final success. In one preparation, a unique type of chromosome element was found at the proximal end of IIR. The nature of this element could not be in doubt: it was in fact the heterochromatic part of the Y-chromosome, hitherto considered invisible. The most striking feature of this structure was its much greater transparency (as compared with the euchromatic portions), with pale bands, which are frequently dotted. In later work, it was often

possible to obtain preparations showing this element, but usually the structure hardly stains at all and could only be seen because of differences in refractive index. However, among a great many preparations, a few were obtained which showed the entire element and many of the features of its structure (see figure 1).

The length of the translocated Y-chromosome portion is about $^3/_5$ or $^1/_4$ of the length of the X-chromosome, or of any of the other elements of the salivary gland chromosomes. Its width is about equal to that of ordinary paired chromosomes. The distal end, or the tip, has no resemblance to any of the other salivary chromosomes of *D. buscki*. The bands are pale, difficult to see, often dotted, and all of them are single (dotting is not a constant character; in some preparations bands appear continuous). The spacing of the bands along the length of the chromosome is about the same as that of the bands of the euchromatic parts of other chromosomes. Because of the generally uniform staining of the individual bands, one gets an impression that they are also more uniformly distributed along the length. It should be noted that observation of the entire length of this chromosome is very difficult, because its visibility depends on the background on which it happens to lie. The best background is a uniform layer of cytoplasm; in places where there is no cytoplasm, the chromosome cannot be seen. By the least pressure on the cover slip, this chromosome is very·easily deformed and disintegrated, leaving no visible trace, indicating an exceedingly fragile structure. Presumably for these reasons it is extremely difficult to observe the structure of the Y-chromosome in individuals without the translocation.

Discussion.—The original concept of heterochromatic chromosomes or chromosome parts as having no structure, concentrated as an amorphous mass in the so-called chromocenter, was radically modified by later investigators. In many chromosomes, genetically inert regions were demonstrated near the centromeres. Prokofyeva[6, 7] showed that such inert regions occurred not only near the centromeres but were also intercalated among euchromatic regions. The same author was first able to observe the Y-chromosome in salivary gland cells.[8] In *D. melanogaster* it can be seen as a short element containing some 8–10 very thin bands. This small Y-chromosome is usually localized at the periphery of the chromocenter, and often pairs with the inert region of the centromere end of the X-chromosome.[8] And yet, according to Prokofyeva, the observed segment of the Y is only its genetically active part, the remainder being invisible. Bauer[10] observed the Y-chromosome of *D. pseudoobscura* as a bundle of chromonemata, with chromioles (the so-called loose heterochromatin). He was unable to see a Y-chromosome in species of *Drosophila* other than *D. melanogaster* and *D. pseudoobscura*. In *D. miranda*, MacKnight[11] demonstrated the Y-chromosome as a complicated structure localized at

the center of the nucleus, together with the chromocenter. The peculiarity of the structure of the Y of this species is that it includes numerous sections of euchromatic material, originally parts of the third chromosome which has become involved in the sex-determining mechanism. These have in the course of time been much modified by fragmentation. Between the euchromatic segments are heterochromatic masses having no definite structure. In the *D. nebulosa*, according to Pavan,[13] the Y-chromosome is represented by a few chromomeres.

The inert part of the Y-chromosome of *D. buschi* which has the definite structure described above is unique. First of all, the translocation having $^1/_3$ or $^1/_4$ of the length of the X-chromosome makes us suppose that the entire Y-chromosome in salivaries is proportionally as long as it is at metaphase; that is, the Y is about equal in length to the X. This conclusion conflicts with the suppositions of Muller and Gershenson[13] and Prokofyeva[9] who suggested that the metaphase Y-chromosome consists of heterochromatic blocks invisible in cells of the salivary glands, or alternatively that it is unspiralized in metaphases. On the other hand the demonstration of a definite structure in the inert Y-chromosome confirms the conclusion of Muller and Prokofyeva[14] concerning the degree of resemblance between euchromatic and heterochromatic segments. We might say that the external appearance of the heterochromatic part of the Y-chromosome in *D. buschi* seems to be a replica of a normal euchromatic chromosome. But these authors made conclusions about the structure of heterochromatic chromosomes in general, on the basis of such small segments as had not yet fully lost genetic activity, and which perhaps for that reason had not yet lost all structural organization. Similarly we might suppose that the Y-chromosome of *D. buschi* is an example of such an element, at an intermediate stage in the process of inactivation.

Two facts are relevant to the above problem. First, the presence in XXY females of an entire Y-chromosome, including its active euchromatic part, in no way modifies their appearance or viability. Second, males lacking almost the entire long left arm of the Y-chromosome are fully viable. Hypoploid males of our mutant line *Curled-Y* thus behave, as far as the viability and the phenotype are concerned, exactly like XO, XY' and XY'' of *D. melanogaster*. One may conclude then that the degree of genetic inactivation of the left arm of the Y-chromosome in *D. buschi* approaches that found in *D. melanogaster*. But the question still remains open why in *D. buschi* the Y-chromosome should still be visible in salivary gland cells even though difficult to observe, while in *D. melanogaster* only an insignificant element of it remains, and in other species are even completely absent.

Summary.—A translocation between the Y-chromosome and an autosome is described in *D. buschi*. The translocation provides a means of

observing in salivary gland preparations the structure of a considerable part of the heterochromatic region of the Y-chromosome. This inert segment resembles a euchromatic chromosome in size and in having clear-cut bands, but it is extremely transparent and takes up almost no stain. The bearing of this on the genetic nature of inert chromosomes is discussed.

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EXPERIMENTS WITH THE CHEMOSTAT ON SPONTANEOUS MUTATIONS OF BACTERIA

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Introduction.—All bacteria require for growth the presence of certain inorganic chemical components in the nutrient, such as potassium, phosphorus, sulphur, etc., and with a few exceptions all bacteria require an energy-yielding carbon source, such as, for instance, glucose or lactate, etc. In addition to these elements or simple compounds, certain bacteria require more complex compounds, for instance an amino acid, which they are not capable of synthesizing. For the purposes of this presentation, any of the chemical compounds which a given strain of bacteria requires for its growth will be called a "growth factor."

In general, the growth rate of a bacterial strain may be within very wide limits independent of the concentration of a given growth factor; but since at zero concentration the growth rate is zero, there must of necessity exist, at sufficiently low concentrations of the growth factor, a region in which the growth rate falls with falling concentration of the growth factor. It therefore should be possible to maintain a bacterial population over an indefinite period of time growing at a rate considerably lower than normal simply by maintaining the concentration of one growth factor—the controlling growth factor—at a sufficiently low value, while the concentrations of all other growth factors may at the same time be maintained at high values.

We shall describe further below a device for maintaining in this manner, over a long period of time, a bacterial population in the growth phase at a reduced growth rate and shall refer to it as the Chemostat.

If the growth rate of a bacterial population is reduced, it is not *a priori* clear whether the growth rate of the individual cells which constitute the population is uniformly reduced or whether a fraction of the total cell population has ceased to grow and is in a sort of lag phase, while the rest keeps growing at an undiminished rate. We believe that under the conditions of our experiments, to be described below, we are dealing with the slowing of the growth rate of the individual cells rather than the cessation of growth of a fraction of the population.

By using an amino acid as the controlling growth factor we were able to force protein synthesis in the bacterial population to proceed at a rate ten times slower than at high concentrations of that amino acid. It appears that we are dealing here with a hitherto unexplored "state" of a bacterial population—a state of reduced growth rate under the control of a suitably chosen growth factor.

The study of this "slow-growth-phase" in the Chemostat promises to yield information of some value on metabolism, regulatory processes, adaptations and mutations of micro-organisms; the present paper, however, is concerned only with the study of spontaneous mutations in bacteria.

There is a well-known mutant of the B strain of coli, B/1, which is resistant to the bacterial virus T_1, sensitive to the bacterial virus T_4, and which requires tryptophane as a growth factor. We used this strain and mutants derived from it in all of our experiments here reported. As a nutrient medium we used a simple synthetic lactate medium (Friedlein medium) with tryptophane added. As the controlling growth factor, we used either lactate or tryptophane.

Experiments on Growth Rates at Low Tryptophane Concentrations.—In order to determine the growth rate of B/1 as a function of the tryptophane concentration (at high lactate concentrations) we made a series of experiments in which we incubated at 37° at different initial tryptophane concentrations *c*, flasks inoculated with about 100 bacteria per cc. and obtained growth curves by determining (by means of colony counts) the number of viable bacteria as a function of time. Because the bacteria take up tryptophane, the tryptophane concentration *c* decreases during the growth of the culture and the growth rate for the initial tryptophane concentration *c* must therefore be taken from the early part of the growth curve.

FIGURE 1

Experiment of September 18, 1950, at 37°C. The curve marked SLOW relates to strain B/1 and the curve marked FAST relates to B/1/f.

The growth rate α is defined by

$$\alpha = \frac{1}{n}\frac{dn}{dt}$$

where *n* is the number of bacteria per cc. The reciprocal value, $\tau = \frac{1}{\alpha}$, we shall designate as the "generation time." From the generation time thus defined, we obtain the time between two successive cell divisions by multiplying by ln 2.

In figure 1 the curve marked "slow" shows the growth rate α as a function

of the tryptophane concentration c for 37°. At low tryptophane concentrations c, the growth rate at first rises proportionately with the concentration; with increasing concentrations, however, the growth rate approaches a limit and for concentrations above 10 γ/l. (micrograms per liter) the growth rate is no longer appreciably different from its highest attainable value. This highest value corresponds to a generation time of $\tau = 70$ min. One half of the highest value is reached at a tryptophane concentration of about $c = 1 \gamma$/l. This concentration corresponds to about three molecules of tryptophane per 10^{-12} cc. (The volume of one bacterium is about 10^{-12} cc.)

The proportionality of the growth rate with the concentration of tryptophane at low concentrations becomes understandable if we assume that the uptake and utilization of tryptophane by the bacterium requires that a tryptophane molecule interact with a molecule of a certain enzyme contained in the bacterium and that the uptake of tryptophane by these enzyme molecules in the bacterium becomes the rate-limiting factor for the growth of the bacterium. On the basis of this argument, we believe that down to as low concentrations of tryptophane as the proportionality of growth rate to concentration can be experimentally demonstrated, the observed growth rate of the bacterial culture represents the growth rate of the individual bacterium and that no appreciable fraction of the population goes into lag.

The Theory of the Chemostat.—In the Chemostat, we have a vessel (which we shall call the growth tube) containing V cc. of a suspension of bacteria. A steady stream of the nutrient liquid flows from a storage tank at the rate of w cc./sec. into this growth tube. The content of the growth tube is stirred by bubbling air through it, and the bacteria are kept homogeneously dispersed throughout the growth tube at all times. An overflow sets the level of the liquid in the growth tube, and through that overflow the bacterial suspension will leave the growth tube at the same rate at which fresh nutrient enters the growth tube from the storage tank.

After a certain time of such operation, at a fixed temperature, a stationary state is reached in the growth tube. We are interested in this stationary state in the particular case in which the growth rate of the bacteria is determined by the concentration in the growth tube of a single growth factor (in our specific case tryptophane). By this we mean that the concentration of a single growth factor (tryptophane) in the growth tube is so low that a small change in it appreciably affects the growth rate of the bacteria, and at the same time the concentration of all other growth factors in the growth tube is so high that a small change in them has no appreciable effect on the growth rate of the bacteria. As we shall show, under these conditions the concentration c of the growth factor in the growth tube *in the stationary state*, for a fixed flow rate w, will be independent of the

concentration a of this growth factor in the nutrient liquid in the storage tank.

In order to see this, we have to consider the following:

1. For zero flow rate of the nutrient ($w = 0$), the bacterial concentration n would rise in the growth tube according to $\frac{1}{n}\frac{dn}{dt} = \alpha(c)$, where α is the growth rate which, according to our premise, is a function of the concentration, c, of the growth factor.

2. In the absence of *growth*, the bacterial concentration in the growth tube would decrease for a given flow rate w according to the formula

$$\frac{1}{n}\frac{dn}{dt} = \frac{-w}{V}$$

where $\frac{w}{V} = \beta$ may be called the "washing-out rate" of the growth tube, and $\frac{1}{\beta}$ the washing-out time.

After a while, for any given flow rate w, a stationary state will be reached · in the Chemostat at which the growth rate α will be equal to the washing-out rate β $\left(\text{and the generation time } \tau \text{ equal to the washing-out time } \frac{1}{\beta}\right)$, i.e.,

$$\alpha(c) = \beta = \frac{w}{V}; \qquad \tau = \frac{1}{\beta} = \frac{V}{w}. \tag{1}$$

Thus, in the stationary state for any fixed flow rate w, the growth rate α is fixed; since α is a function of the concentration c in the growth tube, it follows that c is also fixed and independent of the concentration a of the growth factor in the storage tank.

It may be asked what is the mechanism by which, for different values of a but the same flow rate w, the same concentration c establishes itself in the growth tube in the stationary state. Clearly what happens is this: Suppose that, for a certain concentration a_1 of the growth factor in the storage tank, a stationary state with the concentration c in the growth tube has established itself and subsequently the concentration of the growth factor in the storage tank is suddenly raised to a higher value a_2. When this change is made, the concentration c in the growth tube will at first rise and along with it will rise α, the growth rate of the bacteria, which is a function of c. The concentration of the bacteria in the growth tube will thus start to increase, and therefore the bacteria will take up the growth factor in the growth tube at an increased rate. As the increase of the bacterial concentration continues, the growth rate of the bacteria will, after a while, begin to fall and will continue to fall until a new stationary state is reached

at which the bacteria again grow at the same rate at which they are washed out, i.e., for which again we have $\alpha = \dfrac{w}{V}$. When this state is reached, the concentration of the growth factor in the growth tube is again down to the same value c which it had before the concentration of the growth factor in the storage tank was raised from a_1 to a_2, while the bacterial density is now higher.

In the stationary state the tryptophane balance requires that the following equation hold:

$$a = c + n \frac{V}{w} F(c) \qquad (2)$$

or

$$a = c + n \frac{F(c)}{\alpha(c)} \qquad (3)$$

where $F(c)$ gives in grams per second the amount of the growth factor which one bacterium takes up per second.

As can be easily seen, the amount Q of the growth factor that is taken up per bacterium produced is given by

$$Q = \frac{F(c)}{\alpha(c)}$$

so that, for the stationary state, we may also write

$$a = c + nQ \qquad \text{or} \qquad n = \frac{a - c}{Q} \qquad (4)$$

and for the $c \ll a$ we may write

$$n = \frac{a}{Q}. \qquad (5)$$

The Use of Tryptophane as the Controlling Growth Factor.—Since in the stationary state the tryptophane concentration in the growth tube of the Chemostat is always below 10 γ/1. whenever the generation time is appreciably above 70 min., we may use the approximation given in equation (5) whenever the tryptophane concentration a in the storage tank is above 100 γ/1.

In order to determine the amount of tryptophane, Q, taken up per bacterium produced, we grew bacterial cultures in lactate medium with varied amounts of tryptophane added. We found that if the initial tryptophane concentration is kept below 10 γ/1., then the amount of tryptophane taken up per bacterium produced is not dependent on the tryptophane concentration and has a value of $Q = 2 \times 10^{-11}$ gm. At higher tryptophane concentrations, however, more tryptophane is used up per bacterium produced.

From equation (5), using the value of $Q = 2 \times 10^{-15}$ gm. we obtain $n = 5 \times 10^7$/cc. for $a = 100$ γ/l. and we obtain $n = 5 \times 10^8$/cc. for $a = 1000$ γ/l.

From this, it may be seen that, by choosing suitable values for a and w, we may vary over a wide range, independently of each other, the bacterial concentration n and the tryptophane concentration c.

When we grew B/1 in a Chemostat ($V = 20$ cc.) for ten days at 37° at a generation time of $\tau = 2$ hrs. and at a bacterial density of 5×10^8/cc., we found that a change from the original bacterial strain, B/1, had taken place. The new strain, which we shall designate as B/1/f, differs from the original strain only inasmuch as it grows, at very low tryptophane concentrations, about five times as fast as the original strain. The growth rate at higher tryptophane concentrations is not perceptibly different, nor could we detect any other difference between the two strains. The curve marked "fast" in figure 1 gives the growth rate of the B/1/f strain as a function of the tryptophane concentration at 37°.

The ability of the B/1/f strain to grow faster at very low tryptophane concentrations gives it an advantage over the B/1 strain under the conditions prevailing in the growth tube of the Chemostat; and a mutant of this sort must, in time, displace the original strain of B/1.

Because in our experiments we would want to avoid—as much as possible—population changes of this type in the Chemostat, we used in all of our experiments reported below this new strain, B/1/f.

Spontaneous Mutations in the Chemostat.—If we keep a strain of bacteria growing in the Chemostat and through spontaneous mutations another bacterial strain is generated from it, then the bacterial density n^* of the mutant strain should (for $n^* \ll n$) increase linearly with time, provided that, under the conditions prevailing in the Chemostat, the new strain has the same growth rate as the original strain, so that there is no selection either for or against the mutant. In the absence of selection we have

$$\frac{dn^*}{dt} = \frac{\lambda}{\tau} n \qquad (6)$$

where n^* is the density of the mutant population, n is the density of the population of the parent strain and λ the number of mutations produced per generation per bacterium. Equation (6) holds under the assumption that back mutations can be neglected. From (6), we obtain for $n^* \ll n$

$$\frac{n^*}{n} = \frac{\lambda}{\tau} t + \text{Const.} \qquad (7)$$

From this it may be seen that—as stated above—the relative abundance of the mutants must increase linearly with time if there is no selection for or against the mutant.

If the growth rate of the mutant strain is smaller than the growth rate of the parent strain ($a^* < a$) so that there is selection against the mutant in the growth tube of the Chemostat, then the density n^* of the mutant population should—after an initial rise—remain constant at the level given by

$$\frac{n^*}{n} = \frac{a}{a - a^*} \lambda. \tag{8}$$

Experiments on Spontaneous Mutations in the Chemostat.—Of the various mutations occurring in a growing bacterial population, mutants resistant
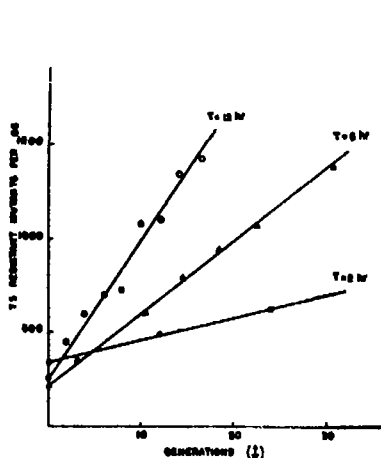
FIGURE 2

Experiments of May 3, 8, and 28, 1950, at 37°C. giving for strain B/1/f for three different values of the generation time the concentration of the mutants resistant to T_5, for a population density of 5×10^8 bacteria per cc.
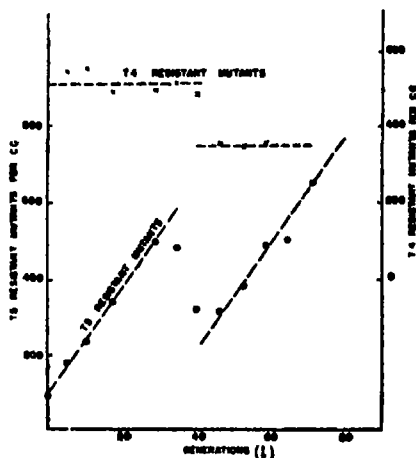
FIGURE 3

Experiment of July 19, 1950, at 37°C. giving for strain B/1/f the concentration of mutants resistant to T_5 (left-hand scale) and mutants resistant to T_4 (right-hand scale) for a population density of 2.5×10^8 bacteria per cc. In this experiment oxygen containing 0.25% CO_2 was used for aeration.

to a bacterial virus are perhaps the most easily scored with considerable accuracy. In our experiments we mostly worked with mutants of our coli strain which were resistant to the bacterial viruses T_5 or T_4.

When we grow the strain B/1/f in the Chemostat with a high concentration of tryptophane but a low concentration of lactate in the nutrient in the storage tank, so that lactate rather than tryptophane is the controlling growth factor, we find—after a short initial period—that the bacterial densities of the mutants resistant to T_5 or T_4 each remain at a constant level. These levels appear to correspond to a selection factor

$\dfrac{\alpha - \alpha^*}{\alpha}$ of a few per cent.

We are inclined tentatively to assume that the behavior of these two mutants exemplifies the general rule that the vast majority of all the different mutational steps leading away from the wild type yield mutants which—under conditions of starvation for the carbon source—grow slower than the parent type.

On the other hand, if we grow our tryptophane-requiring strain in the Chemostat with a high concentration of lactate but a low concentration of tryptophane in the nutrient in the storage tank (so that tryptophane rather than lactate is the controlling growth factor) and if we run the Chemostat at a generation time well above 70 min. (the generation time at high tryptophane concentrations)—then there is no reason to expect mutants *in general* to grow appreciably slower than the parent strain, particularly if the growth of the parent strain is kept very slow by keeping the tryptophane concentration in the growth tube very low. In this case one would rather expect a mutation to affect the growth rate only if it affects the uptake or utilization of tryptophane by the bacterium or if the mutant is a very slow grower. Accordingly, we should, in general, expect the mutant population to increase linearly with time in the Chemostat when tryptophane is used as the controlling growth factor.

Figure 2 gives for 37° the experimental values for the bacterial density for the mutant population resistant to T, in the growth tube of the Chemostat as a function of the number of generations through which the parent strain has passed in the Chemostat. $\left(\text{Number of generations } g = \dfrac{t}{\tau}.\right)$ The three curves in the figure correspond to generation times of 2 hours, 6 hours and 12 hours. The slope of the straight lines gives λ, the mutation rate per generation, as 2.5×10^{-8}; 7.5×10^{-8}; and 15×10^{-8} per bacterium. We see that the mutation rate per generation for $\tau = 6$ hours is three times as high and for $\tau = 12$ hours is six times as high as it is for $\tau = 2$ hours. Thus the mutation rate per generation is, in our experiment, not constant but increases proportionately with τ and what remains constant is the number of mutations produced per unit time per bacterium. According to the above figures, we have $\dfrac{\lambda}{\tau} = 1.25 \times 10^{-8}$ per hour per bacterium.

This result is not one that could have been foreseen. If mutants arose, for instance, as the result of some error in the process of gene duplication, then one would hardly expect the probability of a mutation occurring per cell division to be inversely proportionate to the rate of growth.

If the processes of mutation could be considered as a monomolecular reaction—as had been once suggested by Delbruck and Timofeeff-Ressovsky—then, of course, the rate of mutation per unit time should be

constant.   The rate k of a monomolecular reaction is given by

$$k = Ae^{-W/RT}. \tag{9}$$

The value of the constant A can be calculated from the observed reaction rate k and the heat of activation W (which can be obtained by determining the temperature coefficient of the reaction).

Using the Chemostat, we have determined the rate of mutation to resistance to T_5 at $25°$ (for $\tau = 6$ hrs. and $\tau = 12$ hrs.) and found it to be about one half of the mutation rate at $37°$.   From this value and the mutation rate of $\dfrac{\lambda}{\tau} = 1.25 \times 10^{-8}$ per hour per bacterium at $37°$ we compute $A \approx 10^{-3}$ per sec.

In a condensed system, such as an aqueous solution, A has been found to lie between 10^8 and 10^{14} per sec. for known monomolecular reactions. Therefore if the mutation studied by us were due to a monomolecular reaction, it would have an A value 10^6 times lower than the lowest value so far found.

The density of the mutants resistant to the bacterial virus T_6 in the Chemostat, with tryptophane as the controlling growth factor, also appears to rise linearly with time for $\tau = 2$ hours, $\tau = 6$ hours and $\tau = 12$ hours, but our results so far are not sufficiently accurate to say whether this mutation also occurs at a constant rate per unit time for different generation times τ.   The temperature coefficient of the mutation rate appears to be very low, but again this conclusion must await more accurate experiments.

The result obtained for mutation to resistance to the virus T_5, showing that this mutation occurs at a constant rate per unit time up to a generation time of $\tau = 12$ hours, raises the question whether this is generally true of spontaneous bacterial mutations or whether we are dealing in our case with certain exceptional circumstances.   Clearly, a number of different mutations will have to be examined, different amino acids will have to be used as the controlling growth factor and other conditions will have to be varied before one would draw the far-reaching conclusion that our observation on mutation to resistance to the virus T_5 exemplifies a general rule.

Mutants Resistant to T_4.—We find that mutants resistant to T_4 are selected against in the Chemostat when grown either with lactate or with tryptophane as the controlling growth factor, i.e., the number of mutants remains—after an initial rise—at a fixed level.

It is known that of the different mutants of the B strain of coli which are resistant to the virus T_4, the most frequent one is also resistant to the viruses T_3 and T_7 and that this mutant is a very slow grower under ordinary conditions of culture.   It is conceivable that this might explain why the mutants resistant to T_4 are selected against in the Chemostat even when

the bacterial population grows under tryptophane control and at a much reduced rate.

Manifestation of "Evolution" in the Chemostat.—If a bacterial strain is grown over a long period of time in the Chemostat, from time to time a mutant might arise which grows faster, under the conditions prevailing in the Chemostat, than the parent strain. If this happens, practically the entire bacterial population in the Chemostat will change over from the parent strain to the new strain. We have discussed one change-over of this sort, i.e., the change-over from the strain B/1 to the strain B/1/f. There is no reason to believe, however, that no further change-over may take place when we start out with B/1/f as the parent strain and continue to grow it in the Chemostat over a long period of time.

We have seen that the mutants resistant to T_5 accumulate in the Chemostat and that their number rises linearly with the number of generations, giving a straight line, the slope of which is given by λ. If now at a certain time the population changes over in the Chemostat from the parent strain to a faster-growing strain, the accumulated mutants resistant to the bacterial virus T_5 which were derived from the parent strain should disappear from the Chemostat along with the parent strain. This should lead to a fall in the number of mutants resistant to the bacterial virus T_5 during a change-over from the parent strain to the faster-growing strain. After the change-over to the new strain, the concentration of the mutants resistant to T_5 may be expected again to increase linearly with the number of generations, giving a straight line which has the same slope as before the change-over, because the new strain which displaces the parent strain may be expected to mutate to resistance to T_5 at an unchanged rate λ.

Thus, we may in general expect, when a change-over in the population takes place, the concentration of the mutants resistant to T_5 to shift from one straight line which lies higher to another, which lies lower. The magnitude of this shift may be somewhat different from experiment to experiment, depending on when mutants resistant to T_5 happen to make their first appearance in the population of the new strain.

At the outset, the bacteria belonging to the new strain will be few in number but their number will increase exponentially with the number of generations until—at the time of the change-over—the bacteria belonging to the new strain become an appreciable fraction of the total population. If the mutation rate to resistance to T_5 is of the order of magnitude of 10^{-8}, then it is unlikely that such a mutant should appear in the population of the new strain until its population has reached perhaps 10^7. However, because an element of chance is involved, occasionally a mutant resistant to T_5 may appear earlier and, if that happens, the "shift" associated with the change-over will be smaller and in principle it might even be negative.

If a bacterial population remains growing in the Chemostat for a sufficiently long time, a number of such change-overs might take place. Each

such step in the evolution of the bacterial strain in the Chemostat may be expected to manifest itself in a shift in the ascending straight line curve of the T_5 resistant mutants.

As we have seen, the mutants resistant to T_4 remain—apart from an initial rise—at a constant level in the Chemostat. However, when the bacterial population in the Chemostat changes over from a parent strain to a new strain, the T_4 resistant mutants might change over from one level to another, because the selection against the two strains might be different.

Figure 3 shows, for mutants resistant to T_5 and for mutants resistant to T_4, the number of mutants as a function of the number of generations $\frac{t}{\tau}$ in a Chemostat which was run for 300 hours at $r = 4$ hours with tryptophane as the controlling growth factor.

It may be seen that these two curves show a population change-over of the type just described. The curve for the T_5 resistant mutants shows a shift, P, of $P = 32$ generations.

A number of shifts of this type were observed in different experiments. We verified that these "shifts" represent population change-overs by showing in one case that (under the conditions prevailing in the chemostat) bacteria taken from the Chemostat before the change-over in fact grow slower than bacteria taken from the Chemostat after the change-over.

In order to show this, we took from the Chemostat before the change-over a bacterium resistant to T_5 and after the change-over a bacterium sensitive to T_5 and inoculated a *second* Chemostat (operated under identical conditions) with a 50-50 mixture of these two strains. We then found that the relative abundance of the resistant strain rapidly diminished. In the corresponding control experiment we took a sensitive bacterium from the Chemostat before the population change-over and a resistant one after the population change-over and again found that the strain prevalent before the change-over (this time the sensitive one) was the slower grower.

In the later stages of the change-over the concentration x of the original strain falls off exponentially with the number of generations, $g = \frac{t}{\tau}$, so that we may write $x = Ce^{-t/\tau}$. In our experiment we obtained for γ a value of $\gamma = 3.25$.

It should be noted that the value of γ can be read also directly (though not accurately) from the curve, which gives the concentration n^* of the resistant mutants as the function of g, the number of generations. During the change-over the concentration c of the tryptophane in the growth tube goes over from an initial value c_1 to a final, lower value c_2 and it can be shown that for the midpoint of the change-over at which $c = \frac{c_1 + c_2}{2}$ we have

$$\gamma = \frac{P/4}{1 - \dfrac{1}{\lambda n}\dfrac{dn^*}{dg}} + \frac{1}{2} \tag{10}$$

where P is the magnitude of the shift expressed in the number of generations by which the ascending straight line of the resistant mutants is shifted in the change-over. This formula holds only if τ is large so that the rate of growth of the bacteria in the Chemostat is proportionate to the tryptophane concentration c. Because the exact position on the curve of the midpoint of the change-over on the curve n^* is not known, this formula can give only a rough indication for the value of γ.

In our case, the estimate based on it gave for γ a value of $\gamma = 2.4$ in place of the directly observed value of $\gamma = 3.25$. Within the limits of the accuracy of our curve for n^* these two values are consistent with each other.

Population change-overs manifesting themselves in a shift in the ascending straight line of the T_1 resistant mutants occurred in every experiment carried at $\tau = 4$ hrs. beyond the 50th generation. In an experiment carried to the 450th generation at a bacterial density of 2.5×10^8/cc., a number of such shifts occurred, the last one at about the 350th generation. (In the course of this experiment the mutants resistant to T_1 rose twice from a low level to a high peak, the first of which reached 4.6×10^4 and the second 4.5×10^4 mutants per cc. This phenomenon is now being investigated.)

It may be said that our strain, if grown in the Chemostat at low tryptophane concentration for a long period of time, undergoes a number of mutational steps, each one leading to a strain more "fit" than the previous one, and that each step in this process of evolution becomes manifest through the shifts appearing in the curve of the mutants resistant to T_1.

THE ANALYSIS OF A CASE OF CROSS-STERILITY IN MAIZE

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The male gametophyte of maize is extremely sensitive to chromosomal unbalance. Duplications and deficiencies are almost always accompanied by reduced pollen transmission. The functioning of the male gametophyte is also known to be affected by a number of genic factors. The most thoroughly studied locus and probably the most interesting is Ga_1 on chromosome 4. First detected by Correns[3] because of aberrant F_2 ratios for sugary-starchy, this factor was subsequently studied by Jones,[4,10]

Emerson,[6,7] Mangelsdorf,[10] and Demerec.[4]   Emerson established that the differential fertilization was not due to the sugary gene itself but to some other gene with which it was linked.

Ga_1 is comparable to the self-sterility genes found in strictly cross-fertilized plants such as Nicotiana, Oenothera, Trifolium, etc., in that there is interaction between the stylar tissue and the male gametophyte.   When the female parent is homozygous recessive ga_1, ga_1 pollen can compete successfully against Ga_1 and fertilize half of the ovules.   However, if the plant used as the egg parent is heterozygous or homozygous Ga_1, the ga_1 pollen is a poor competitor and achieves fertilization in only 0–4% of the ovules.   In the absence of competition, that is, if only ga_1 pollen is used in the cross, full seed set is obtained regardless of the genotype of the female parent.[7]   Thus the incompatibility between ga_1 pollen and Ga_1 silk is not detectable in the absence of competing Ga_1 pollen.   The purpose of this paper is to present evidence for the existence of a third allele at this locus.

TABLE 1

SUMMARY OF DATA SHOWING THE COMPETITIVE FUNCTIONING OF $Ga_1{}^s$, Ga_1, AND ga_1 ON STYLES OF VARIOUS GENOTYPES

ITEM	GENOTYPIC CONSTITUTIONS	NUMBER OF KERNELS			% SUGARY
		STARCHY	SUGARY	TOTAL	
1	$Ga_1{}^sSu/Ga_1su \times Ga_1{}^sSu/Ga_1su\,\male$	19,168	5865	25,033	23.4
2	$Ga_1{}^sSu/ga_1su \times Ga_1{}^sSu/Ga_1su\,\male$	4,194	1300	5,494	23.6
3	$Ga_1su/Ga_1su \times Ga_1{}^sSu/Ga_1su\,\male$	781	666	1,447	46.0
4	$Ga_1su/ga_1Su \times Ga_1{}^sSu/Ga_1su\,\male$	4,624	1432	6,056	23.6
5	$Ga_1{}^sSu/Ga_1su \times Ga_1{}^sSu/ga_1su\,\male$	1,548	221	1,769	12.5
6	$Ga_1{}^sSu/ga_1su \times Ga_1{}^sSu/ga_1su\,\male$	4,142	611	4,753	12.9
7	$Ga_1su/Ga_1su \times Ga_1{}^sSu/ga_1su\,\male$	237	86	323	26.6
8	$Ga_1su/ga_1Su \times Ga_1{}^sSu/ga_1su\,\male$	3,866	675	4,541	14.9
9	$ga_1su/ga_1su \times Ga_1{}^sSu/ga_1su\,\male$	412	408	820	49.8
10	$Ga_1{}^sSu/ga_1su \times Ga_1su/ga_1Su\,\male$	2,096	1104	3,200	34.6

This allele, designated $Ga_1{}^s$ since its effect is stronger than Ga_1, was found by Dr. M. M. Rhoades who turned it over to the author for further analysis.

The most important difference between Ga_1 and $Ga_1{}^s$ is that ga_1 pollen completely fails to function on styles homozygous for $Ga_1{}^s$ even in the absence of competing pollen.   From 14 such crosses only one seed was obtained; this could have been the result of contamination.   Crosses of ga_1 pollen on styles heterozygous for $Ga_1{}^s$ yield a partial seed set.   Less seed is set when the female parent is heterozygous $Ga_1{}^sGa_1$ than when a heterozygous $Ga_1{}^sga_1$ plant is used.   The total amount of seed produced in such crosses varies greatly from plant to plant.

The linkage relation of $Ga_1{}^s$ with Su and its interaction with Ga_1 suggest that it is an allele of Ga_1.   Both Ga_1 and $Ga_1{}^s$ exhibit selective fertilization in competing with ga_1 only when the female parent in the cross carries one of the dominant alleles.   No selective fertilization occurs when the female

parent is homozygous ga_1 (table 1, item 9).   On the assumption that Ga_1 and $Ga_1{}^s$ represent two independent loci, and that ga_1 and ga^s gametes can compete successfully on respective recessive styles against pollen carrying the dominant allele, no differential fertilization should result from crosses of $ga_1ga_1Ga^sGa^s$ by $Ga_1ga_1ga^sga^s{}^{\sigma}$ and of $Ga_1Ga_1ga^sga^s$ by $ga_1ga_1Ga^sga^s{}^{\sigma}$. Moreover, pollen from $Ga_1Ga_1ga^sga^s$ individuals should give no seed set when crossed on $ga_1ga_1Ga^sGa^s$ plants.   This, however, was not found to be true; in the crosses of $Ga_1{}^sga_1 \times Ga_1ga_1{}^{\sigma}$ and $Ga_1{}^sGa_1 \times Ga_1{}^sga_1{}^{\sigma}$, ga_1 pollen is a very poor competitor (table 1).   The cross $Ga_1{}^sGa_1{}^s \times Ga_1Ga_1{}^{\sigma}$ yielded a full seed set.   These data negate the hypothesis that $Ga_1{}^s$ and Ga_1 represent two independent loci but do not rule out the possibility of an interaction between the two.   One might propose that the presence of Ga_1 in the Ga_1ga^s gamete permits the functioning on Ga^s styles of an otherwise handicapped pollen grain.   It seems unlikely, however, that such inter- action of loci would exist in view of the specific action of the self-sterility alleles in Nicotiana.   East and Yarnell[5] have shown that there are at least 15 alleles at the self-sterility locus.   Each allele is so specific in its action that the rate of growth of a pollen tube containing any one of them is determined solely by the presence of that particular allele in the diploid sporophytic tissue of the style and is completely independent of any other which may also be present.   This specificity breaks down to some extent in polyploids of Oenothera[9] and white clover.[1]

' Linkage studies have shown that the recombination values obtained for $Ga_1{}^s$ and Su are very close to those found by Emerson for Ga_1 and Su.   A recombination value of 30% between the latter was calculated on the basis of 0% functioning of ga_1 pollen.   This value was obtained by determining the percentage of sugary kernels resulting from a cross such as $Ga_1su/Ga_1su \times Ga_1Su/ga_1su{}^{\sigma}$.   If no ga_1 pollen functions, all of the sugary kernels result from crossing over between Ga_1 and Su, and the percentage of sugary kernels on the ears represents the crossover frequency.   However, if some ga_1 pollen does function, then not all of the sugary kernels are crossovers and the recombination value is somewhat lower.   Emerson showed that ga_1 pollen is responsible for the fertilization of from 0 to 9% of the ovules with the mean at 4%.   On the basis of 4% functioning of ga_1 pollen he calcu- lated a recombination value of 27.8%.   When the $Ga_1{}^s$ allele was sub- stituted for Ga_1 in similar crosses, a percentage of 25.8% sugary kernels was obtained (table 1, item 6).   This value, which represents the recom- bination between $Ga_1{}^s$ and Su, agrees well with that calculated by Emerson for Ga_1-Su.

That the discrepancy between the recombination values of 30% for Ga_1-Su and 25.8% for $Ga_1{}^s$-Su is due to the amount of ga_1 pollen which func- tions is best shown by the following experiment. $Ga_1{}^sSu/ga_1su$ plants were used as the pollen parents in crosses with (1) Ga_1su/ga_1Su, and (2)·

$Ga_1{}^sSu/ga_1su$ plants. Since the same male parent was used in both crosses the amount of crossing over between $Ga_1{}^s$ and Su would be identical and any significant difference in the percentage of sugary kernels resulting from these crosses would be due to the functioning of ga_1 pollen. The data are given as items 6 and 8 in table 1. The crosses involving Ga_1 gave 14.9% sugary kernels while 12.9% sugary seeds were obtained when $Ga_1{}^s$ was involved. The difference between 12.9 and 14.9% is significant at the 1% level and may logically be ascribed to the differential functioning of ga_1 pollen on Ga_1 and $Ga_1{}^s$ silks. The value of 14.9% is very close to the 15.1% found by Emerson in similar crosses using Ga_1. It follows that ga_1 pollen occasionally functions on Ga_1 styles even when competing against $Ga_1{}^s$ pollen.

The close agreement in the amount of crossing over between $Ga_1{}^s$ and Ga_1 with Su is indicative of an allelic relationship between the two genes. However, a possible alternative must be considered—namely, that $Ga_1{}^s$ and Ga_1 represent two loci lying equidistant from (in terms of crossover units), but on opposite sides of, Su. Such a condition would place the two genes more than 50 map units apart.

TABLE 2

DISTRIBUTION OF EARS RESULTING FROM THE SELF-POLLINATION OF PLANTS DERIVED FROM THE CROSS $Ga_1{}^sSu/Ga_1su \times ga_1su\,\male$

FAMILY	SUGARY KERNELS, %			
	8–12	13–17	18–22	23–27
1360	0	4	3	0
1361	6	24	7	0
1362	1	7	3	0

In order to test this hypothesis the following crosses were made: $(Ga_1{}^sGa_1{}^sSuSu \times Ga_1Ga_1susu\,\male) \times ga_1ga_1susu\,\male$. The sugary seeds were discarded since a heterozygous Su/su condition is necessary to detect the presence or absence of the gamete factor. On the assumption that two loci are involved, the F_1 would be genotypically ga_1SuGa^s/Ga_1suga^s. The second cross would yield four classes of starchy kernels: (1) ga_1SuGa^s/ga_1suga^s, (2) Ga_1SuGa^s/ga^ssuga_1, (3) ga_1Suga^s/ga^ssuga_1, and (4) Ga_1Suga^s/ga^ssuga_1. The first class is a non-crossover; the second, a single crossover in region (1) $(Ga_1\text{-}Su)$; the third, a single crossover in region (2) $(Su\text{-}Ga^s)$; and the fourth represents a double crossover in regions (1) and (2). Classes 1, 2 and 4 are difficult to distinguish and when self-pollinated would yield only 12–15% sugary seeds due to the linkage of Su with the dominant gamete factor. However, class 3, comprising almost 25% of all the starchy seeds would be detected since in the absence of either Ga_1 or Ga^s normal Mendelian ratios of starchy-sugary kernels, i.e., 25% sugary, should result upon self-pollination. On the other hand, if Ga^s and Ga_1, are alleles of the same locus, the cross of the F_1 with ga_1su should give only 2 classes of

starchy seeds, $Ga_1{}'Su/ga_1su$ and Ga_1Su/ga_1su. On this basis, no 3:1 starchy-sugary ratios would be expected on self-pollinated ears. The results are summarized in table 2. Since only low sugary ratios resulted, it is apparent that $Ga_1{}'$ and Ga_1 are not separate loci located on opposite sides of Su.

When Ga_1 and $Ga_1{}'$ pollen compete, the latter has a definite advantage in achieving fertilization on both Ga_1 and $Ga_1{}'$ styles. Tests to determine the degree of selective fertilization were made using female parents of four genetic constitutions: (1) $Ga_1{}'Su/Ga_1su$, (2) $Ga_1{}'Su/ga_1su$, (3) Ga_1su/Ga_1su and (4) Ga_1su/ga_1Su. The pollen parents were $Ga_1su/Ga_1{}'Su$. The results are given in table 1. In the absence of any differential fertilization the crosses with the heterozygotes should produce 25% sugary kernels and the backcrosses to homozygous sugary plants should yield equal numbers of both types of seeds. However the 23.5% sugary kernels in the F_2 population and the 46% sugary in the backcrosses were significantly different at the 1% level from the expected percentages. Assuming 26% recombination between Ga_1 and Su it may be calculated that $Ga_1{}'$ pollen fertilized 56% of the ovules in competition with Ga_1 pollen.

The manner in which selective fertilization is accomplished has not been established. Jones,[3] working with Ga_1, compared the ratios of sugary to starchy kernels on the butt and tip ends of the ears and concluded that the causal agent was differential pollen-tube growth. Emerson[7] was not able to confirm this. Demerec[4] claimed that selective fertilization was due to cross sterility rather than to competition between the pollen tubes. However, from his data it would appear that Demerec was working with a different allele from that studied by Jones and Emerson. Demerec's gamete factor is analogous to the $Ga_1{}'$ allele reported in this paper in that ga_1 pollen does not function well in styles which carry the dominant allele even in the absence of competing pollen. The 12.4% sugary kernels which he obtained in the F_2 of sweet corn by plants showing the cross-sterility compares well with the reported 12.9% sugary resulting from the self-pollination of $Ga_1{}'Su/ga_1su$ plants.

Demerec listed three possible explanations of this cross sterility.

(1) Inability of pollen with recessive gametophyte factor to germinate on silks having dominant allele.

(2) Inability of recessive pollen tubes to grow in silks with dominant allele.

(3) Inability of recessive pollen tubes to reach the ovules in the plant having the dominant allele.

In the case of $Ga_1{}'$, it was possible to eliminate the first two causes experimentally. Plants of the constitution $Ga_1{}'Ga_1{}'$ were pollinated with ga_1 pollen. Two hours after pollination the silks were removed, fixed in modified Carnoy solution and stained with propionic carmine. It was evident

that the pollen grains had germinated and that the pollen tubes had penetrated the stylar tissue.

Demerec's third suggestion remains as a possibility and a fourth may be added which is likewise untested. The failure of ga_1 pollen to fertilize $Ga_1{}^s$ plants may be independent of stylar interaction altogether, and may involve some abnormal reaction within the ovule, such as inability of the pollen tube to penetrate the embryo sac. None of these explanations, however, are applicable in the case of Ga_1-ga_1 competition since, in the absence of Ga_1 pollen, ga_1 gametes function normally on Ga_1 styles.

A number of cases of cross-sterility in maize may be due to the action of $Ga_1{}^s$. This is especially true of the cross-sterility found among varieties of popcorn.[2] Although the point of origin of the $Ga_1{}^s$ allele is not known, the fact that both Ga_1 and Demerec's cross sterility factor were found in popcorn supports this contention.

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THE USE OF SODIUM NUCLEATE IN THE STUDY OF THE MUTAGENIC ACTIVITY OF ACRIFLAVINE IN ESCHERICHIA COLI*

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In a previous study,[1] neutral acriflavine was shown to induce mutations from phage-sensitivity to phage-resistance in *Escherichia coli*. The present report concerns an improved method of investigating the mutagenic potency of certain compounds, using acriflavine as a model.

One of the difficulties encountered in working with acriflavine, as well as with numerous other compounds, was the tenacity with which the chemical remains bound to the treated cell. Ordinary washing methods were effective in removing acriflavine only to a limited degree, and thoroughly washed suspensions of treated bacteria were found to contain enough active chemical to limit subsequent growth, and to interfere with

the process of infection by bacteriophage. Another problem was the tendency of acriflavine to cause clumping of bacteria in dense cell suspensions. The agglutination could be controlled in part by adjustment of experimental conditions, but was extremely difficult to eliminate entirely. Since these complications, which were encountered with several other compounds, could operate to simulate mutagenicity, it seemed important to develop a method whereby they could be circumvented or overcome.

It has been reported that the antibacterial activity of acridines can be inhibited by the addition of nucleic acids and some of their derivatives,[2,3] and the kinetics of this interaction have been described.[4] In these experiments, sodium ribonucleate was used to bring the exposure of bacteria to acriflavine to a sharp end point, to eliminate residual activity of this substance in the subsequent handling of the treated bacteria, and to prevent agglutination. In principle, this method is applicable to other antibacterial compounds for which more or less specific reversing agents are available.

Material and Methods.—Strain B/r of *E. coli* was used. The stock was carried on nutrient agar slants, and subcultured every two months. Inocula for experimental cultures were taken from a 24-hour culture grown in nutrient broth from about 100 cells.

Bacteriophage T1 was used in the isolation of mutants, and was kept in broth suspensions having a titer of about 10^{10} particles per ml.

Difco nutrient broth and Difco nutrient agar, each with 0.5% NaCl added, were used as culture media.

Neutral acriflavine was obtained from the National Aniline Division of the Allied Chemical and Dye Corporation, New York City. Solutions were made in distilled water, sterilized by boiling and used for no longer than three days. Sodium ribonucleate (yeast), obtained from Schwartz Laboratories, New York City, was dissolved in distilled water and sterilized by immersion in a boiling water-bath for fifteen minutes.

All assays were made by quantitative dilution and surface plating. Determinations of the frequency of phage-resistant mutants were made by spreading 0.1-ml. aliquots of culture on the surface of plates previously coated with about 10^9 phage particles.

Incubation was at 37°C.

Each experimental series consisted of 20–50 tubes containing 5 ml. of broth to which acriflavine was added to give a concentration of 0.01%. Each tube was inoculated with about 10^7 bacteria and incubated for 3–4 hours, or until survival would be expected to reach between 10^{-2} and 10^{-3}. At this time, sodium nucleate was added to each tube, to give a concentration of 0.5%, and several tubes were assayed immediately to determine precisely the number of viable bacteria per culture. The cultures were then incubated for 18–24 hours, after which several were assayed

to determine the average titer, and an aliquot from each culture was plated with phage to obtain the number of T1-resistant mutants.

Controls consisted of 20–50 tubes containing 5 ml. of 0.01 acriflavine in broth, to each of which were added *simultaneously* sodium nucleate (final concentration 0.5%) and an inoculum of 10^4–10^5 untreated bacteria. These cultures were incubated 18–24 hours, and assayed for titer and phage-resistant mutants in the same manner as the experimental cultures.

Thus the experimental and control cultures were grown from inocula of comparable size in a medium containing both acriflavine and nucleate. In the case of the experimental cultures, the inoculum consisted of sur-

FIGURE 1
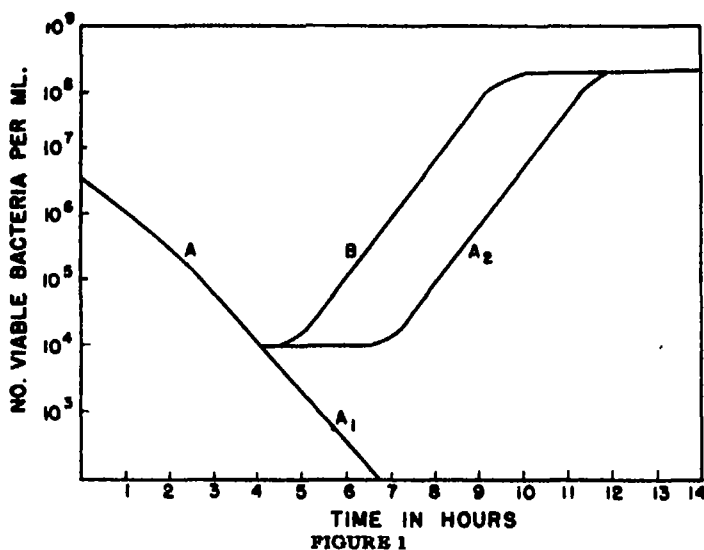
Killing and Growth in Experimental and Control Cultures. A-A_1 = killing curve of B/r in 0.01% acriflavine in broth; A_2 = growth of acriflavine-treated bacteria after addition of 0.5% sodium nucleate (experimental cultures); B = growth of untreated bacteria in broth containing 0.01% acriflavine + 0.5% sodium nucleate (controls).

vivors of a prior exposure to acriflavine alone, whereas in the controls untreated bacteria were used.

Experimental Results.—Growth in Nucleate-Inactivated Acriflavine: Figure 1 shows the course of killing and growth in the experimental cultures, and of growth in the controls. Killing proceeds logarithmically in acriflavine until the addition of nucleate, at which time it is sharply arrested. The growth of the survivors in the nucleate-acriflavine mixture is comparable to that of the controls, except for the prolongation of the lag phase. The final titers of experimental and control cultures were not significantly different.

Frequency Distribution of Phage-Resistant Mutants in Experimental and Control Cultures.—Figure 2 shows the frequency distribution of mutants resistant to bacteriophage T1 in 300 experimental cultures and 300 control cultures. The spread observed in the control series is a function of the spontaneous mutation rate and the clonal grouping of mutants in liquid cultures,[5] and has been found to be extremely reproducible. It will be seen that the distribution of mutants in the experimental cultures is very different. The largest class of cultures in the experimental series is that having between 10^2 and 10^3 mutants per 10^8 bacteria, whereas the largest control class has less than 10 mutants per 10^8 bacteria. The frequency distributions for both experimental and control culture series are highly

MUTANTS PER 10^8 BACTERIA

FIGURE 2
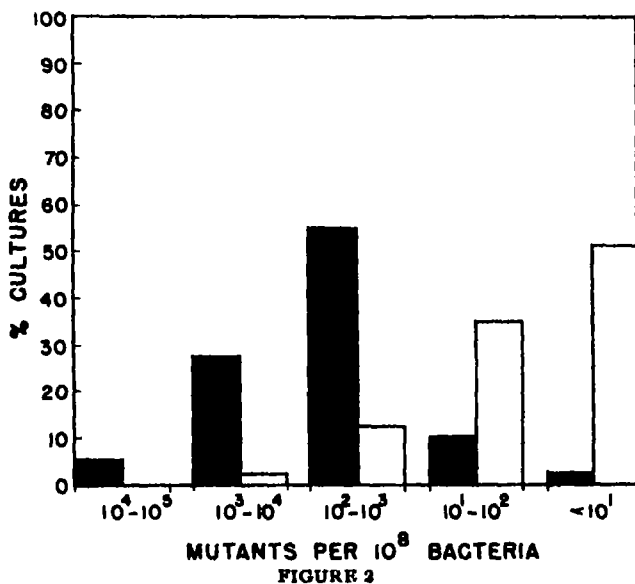
Frequency distribution of T1-resistant mutants for 300 experimental cultures (black columns) and 300 control cultures (white columns).

reproducible under the conditions specified, as will be shown below in another connection.

Selection.—The observed shift in the frequency distribution of mutants in experimental cultures can be accounted for by induced mutation only if two possible modes of selection can be ruled out. Selection could enter through differential survival of spontaneous mutants in the experimental cultures during exposure to acriflavine, or through differential stimulation of the rate of growth of phage-resistant mutants by the acriflavine-nucleate mixture, or through a combination of the two. These possibilities were tested in the following way: Ten single-colony isolates of phage-resistant

mutants were made, each colony coming from a plating on phage of a different experimental culture. If selection were responsible for the increased frequency of mutants in these cultures, mutants thus obtained should possess the particular advantages involved, having been through the selective sieve of the experimental procedure. After repeated streaking and single-colony isolation to eliminate contaminating phage particles, the ten phage-resistant strains were grown in broth. Mixtures were made of each mutant strain with the phage-sensitive parent strain, and each mixture was assayed to determine the relative proportions of its components. The mixtures were then subjected to the experimental procedure described above: inoculation into acriflavine, and incubation for four hours followed by addition of sodium nucleate and incubation for 18–24 hours. Assays to deter-

TABLE 1

SELECTION TEST—ARTIFICIAL MIXTURES OF 10 INDEPENDENT T1-RESISTANT MUTANT STRAINS AND B/r, SUBJECTED TO THE EXPERIMENTAL PROCEDURE

M_o, per cent Initial proportion of T1-resistant mutants

M_e, per cent Proportion of T1-resistant mutants immediately after the addition of sodium nucleate

M_f, per cent Final proportion of T1-resistant mutants.

B/r/1 STRAIN	M_o, %	M_e, %	M_f, %	B/r/1 STRAIN	M_o, %	M_e, %	M_f, %
1	1.3	1.9	0.6	6	0.6	0.8	0.3
	15.3	18.1	9.4		8.6	6 2	4.1
2	1.1	1.3	0.7	7	0.9	0.8	0.4
	10.3	8.6	9.1		23.1	32.6	19.3
3	1.4	1.4	1.5	8	1 5	2 0	1.8
	22.4	17.4	20.2		13.7	10 1	15.4
4	0.7	0.9	1.0	9	0.5	0.8	0.8
	14.5	10.7	20.1		19 3	29.2	14.6
5	1.1	0.9	0.8	10	3.0	2.1	1.8
	46.0	33.2	35.6		36.8	56.6	42.0

mine the proportions of phage-resistant and phage-sensitive components were made immediately after the addition of nucleate, and again after the 18- to 24-hour incubation period. The results are shown in table 1. It is evident that selection does not favor the phage-resistant mutants, either by differential survival or by differential growth.

Mutagenic Effect of Acriflavine on an Acriflavine-Resistant Strain.—The growth of the normal B/r strain is completely inhibited by a concentration of 0.002% of neutral acriflavine in broth. By serial transfer in increasing concentrations of acriflavine, a strain was derived from B/r which could grow in 30 times this concentration, or 0.06%. This acriflavine-resistant strain, when treated with acriflavine according to the standard procedure described above, using a concentration of 0.01% acriflavine, gave a frequency distribution of phage-resistant mutants identical with

that of the untreated controls.   The four-hour exposure to this concentra-
tion of acriflavine was entirely non-toxic for the resistant strain.   When the
experiment was repeated, however, using a concentration of 0.3%, which
gave a survival of 10^{-2} to 10^{-3}, the frequency distribution of phage-resist-
ant mutants was essentially the same as that obtained with strain B/r at
the 0.01% concentration, which yields the same survival during the 4-hour
treatment.   Figure 3 shows the frequency distributions of phage-resistant
mutants obtained for the acriflavine-resistant strain, using 0.3% acri-
flavine, as compared with those obtained for B/r at a concentration of
0.01%, and for untreated controls.   It is evident that the mutagenic effect
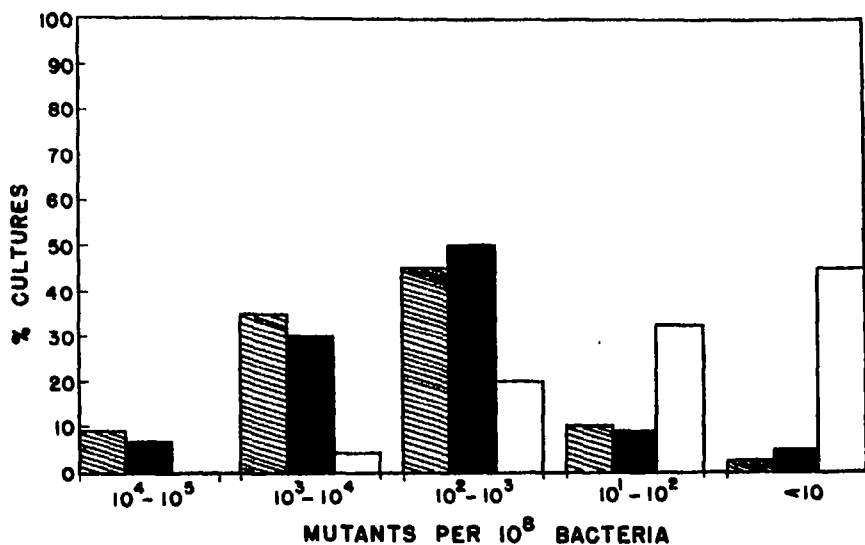
FIGURE 3

Frequency distribution of T1-resistant mutants for 100 experimental cultures of
acriflavine-resistant bacteria (cross-hatched columns);   100 experimental cultures of
acriflavine-sensitive bacteria (black columns);   and 100 controls (white columns).

of acriflavine is related to the survival levels of the sensitive and resistant
strains, rather than to the absolute concentration of acriflavine used in the
treatment.

Discussion.—The mutagenicity of acriflavine is confirmed in these ex-
periments, under conditions free of the complications encountered earlier
and enumerated above.   The method is essentially qualitative, since the
cultivation following the acriflavine treatment introduces clonal variance,
which makes precise determination of the frequency of induced mutations
extremely difficult.   In addition, the method as described does not permit
distinction between "zero-point" and delayed mutations, and both types
could contribute to the greater frequency of cultures having high numbers

of mutants. Modifications that would permit the separation of the two types of induced mutations are theoretically possible, however. One such procedure would involve adding bacteriophage to the nucleate-inactivated acriflavine tubes immediately after adding nucleate, so that only tubes containing at least one zero-point mutant would develop turbidity. Addition of bacteriophage after various incubation periods would similarly permit the measurement of delayed mutations. Preliminary experiments along these lines have been done, with promising results.

The observation that the acriflavine-resistant strain requires a much higher concentration of acriflavine than the original strain to produce a given level of mutagenic activity is of interest in relation to a similar comparison made by Demerec and Latarjet.[6] These authors found that strains B and B/r, which are, respectively, sensitive and resistant to ultra-violet radiation,[7] give the same yield of induced phage-resistant mutants at a given dosage of ultra-violet, regardless of the wide difference in survival. The apparent contradiction between these observations may be spurious, however. It is possible that the resistance of the acriflavine-resistant strain may depend upon a permeability change, or upon some other property whereby the amount of acriflavine present inside the cell is reduced. The survival level may be a measure of the effective concentration within the cell, in which case the correspondence between levels of survival and mutagenic effect would not be surprising.

It should be mentioned that in using this procedure it is necessary to adjust conditions so as to obtain survivals in the range indicated (10^{-2} to 10^{-3}). Survivals outside this range rarely give positive results, as there seems to be a delicate interplay between the killing rate and the rate of induced mutation. For any other compound, the optimal survival range must be determined empirically.

Summary.—(1) A method is described whereby exposure of bacteria to the action of acriflavine can be sharply arrested, and its residual activity eliminated by the addition of sodium nucleate.

(2) The mutagenic activity of acriflavine, as measured by its ability to induce mutations from phage-sensitivity to phage-resistance in strain B/r of *E. coli*, is confirmed by the use of the nucleate-reversal technique.

(3) An acriflavine-resistant strain of *E. coli*, derived from B/r, requires 30 times as much acriflavine as does B/r to give a comparable mutagenic effect, as well as a comparable level of survival.

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THE NATURE OF BONE AND PHOSPHATE ROCK

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Carbonate has never been adequately explained as a constituent of bone, dentine, enamel, and the closely related phosphorites. Its failure to act quite like $CaCO_3$, upon heating for instance, has, since the time of Hoppe-Seyler[1] led to postulated calcium carbonate phosphate compounds as essential constituents of bone or enamel. Existence of optically homogenous apatites such as the minerals francolite[2] and collophane containing carbonate apparently supports this conclusion and has led to the acceptance of an apatite structure containing carbonate.

Preferential solution by acid of carbonate in bone has often been noted[3-5] and has been interpreted by some workers[4] as evidence for the presence of $CaCO_3$ as a separate phase. Crystalline $CaCO_3$, however, has not been observed in bone by the very sensitive test of its high birefringence. A Geiger-counter x-ray spectrometer, moreover, gives no evidence of its presence, while one-third as much calcite as would correspond to the analysis can be detected when mixed with hydroxylapatite, or naphtha-extracted bone. More incontrovertible evidence is afforded by the work of Logan and Taylor[5] which shows that an apatite phase will precipitate, from a solution having the inorganic composition of plasma, in the presence of carbonate and steadily withdraw CO_3^{--} from a solution unsaturated with respect to $CaCO_3$.

Bone, enamel, dentine and collophane, the last being the apparently isotropic component of phosphate rocks, give rather diffuse x-ray powder diffraction patterns identical, within their definition, with that of fluorapatite.[6] Francolite, the more highly birefringent material of phosphorites, can be obtained as optically clear single crystals containing more than 3% CO_3.[3] X-ray goniometer photographs of francolite crystals are identical with those of fluorapatite, even in very high orders of interference maxima. From the structural point of view it seemed necessary for a large number of CO_3 groups to be present in an apatite structure without changing the diffraction pattern, even in minor ways, which is very unlikely.

The average inorganic compositions of human bone, and enamel, based upon a great number of published analyses, can be represented by the following formulas[7] in which the number of positive ions is taken as 10.0:

Bone $[Ca_{9.80}Mg_{0.33}Na_{0.33}][(PO_4)_{5.06}(CO_3)_{1.38}][H_2O]_x$

Enamel $[Ca_{9.70}Mg_{0.19}Na_{0.11}][(PO_4)_{5.80}(CO_3)_{0.45}][OH]_{1.57}[H_2O]_x$
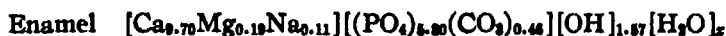
Three quite different suggestions for the structural nature of the compound present have been advanced in recent years. Trömel and Möller[3] and Bale and coworkers[4, 9] consider the material to be hydroxylapatite, $Ca_{10}(PO_4)_6$-$(OH)_2$, with "adsorbed" carbonate and possibly phosphate. Bale[6] states "Since the diffraction pattern of tooth and bone substance give no indication of a carbonate apatite being present, the carbonate ions are also probably most abundant in these regions of structural discontinuity that may be considered as marking the transition from one crystalline particle to another." Brasseur and Dallemagne in a series of papers published in Belgium during the war which were summarized in *Nature* in 1946[10] (also reference 4) concluded that bone is "tricalcium phosphate hydrate," $Ca_9(PO_4)_6(H_2O)_2$, with $CaCO_3$ present as a separate phase and that enamel is in part "carbonate hydroxylapatite." They state "We cannot admit, therefore, that the tertiary with a Ca/P ratio of 1.94 is hydroxylapatite with adsorbed PO_4 ions." Hendricks and Hill in 1942[7] reached essentially these same conclusions except that they considered the carbonate of bone to be present in the "tricalcium phosphate hydrate" lattice.

Properties of the several materials pertinent, without further discussion, to the question at issue are:

1. A single phase having the x-ray diffraction pattern of apatite and containing carbonate is present even though bone forms in a system unsaturated with respect to calcium carbonate.

2. In human bone this phase is shown by analysis to be a neutral substance. It is more basic, approaching hydroxylapatite, in parts of enamel.

3. A large portion of the carbonate in bone can be preferentially removed by solution in acid without destruction of the apatite lattice. Enamel and some "carbonate apatites" dissolve uniformly. .

4. Citrate is present in bone as water insoluble form in amounts by volume approaching one-half that of carbonate.[11, 12]

The first and third of these properties indicate rather clearly that carbonate is on the surface of the bone material. Presence of citrate can also be explained in this way instead of being merely "adventitious." Bone also contains magnesium and sodium that might be expected to replace Ca^{++} in the apatite structure on the basis of ionic radii for Na^+ and Mg^{++}. These two are preferentially dissolved by acid,[8] however, indicating their location on the lattice surface. The difficulties of charge with

Na$^+$ replacing Ca^{++} and essential absence in solutions near neutrality of magnesium as Mg^{++}, rather being Mg(OH)$^+$, accordingly do not arise. There is still a size limitation for the surface, however, since Na$^+$ is taken up preferential to K$^+$.

The nature of "carbonate apatites" has been most confusing in its bearing on the type of compound present in bone. Lack of preferential solution in acid of carbonate in enamel was essentially the reason for Brasseur and Dallemagne considering the compound to be "carbonate hydroxylapatite." Another possibility, however, is that the carbonate groups of enamel and francolite and the citrate of enamel are also present
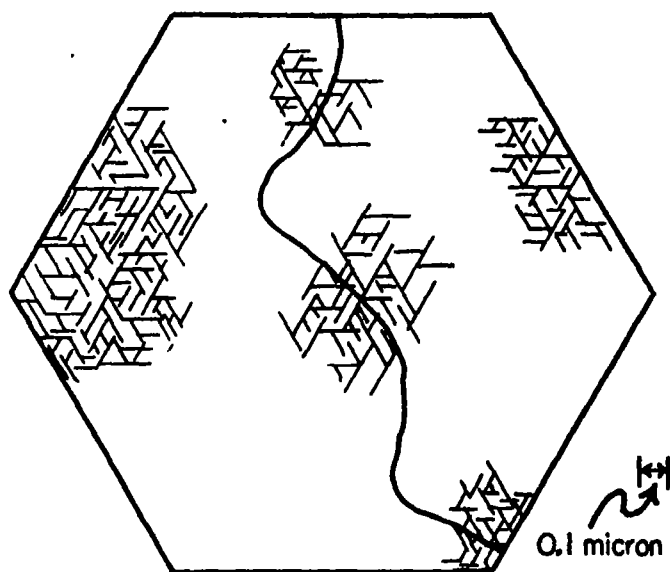
FIGURE 1

Entrapped surfaces in "carbonate apatites" as present in enamel
and francolite, schematic.

on surfaces which are entrapped as crystallites grow and are surrounded by phosphate such that the final material has many occluded surfaces as schematically shown in figure 1. In the case of francolite, and to a lesser extent in enamel, the apatite lattice can be continuous throughout large crystals. The high density of francolite[2] indicates that voids cannot constitute more than 1% of the total volume.

Evidence in regard to entrapped surfaces for francolite and collophane comes from some peculiarities of their fluorine contents which have not previously been explained. These substances[3, 13] contain fluorine in excess of that required for an apatite structure, sometimes by a factor as great as

1.50, while fluorapatite has not been found to contain excess fluorine. Now if the surface occurs at primitive translations and all fluorine positions
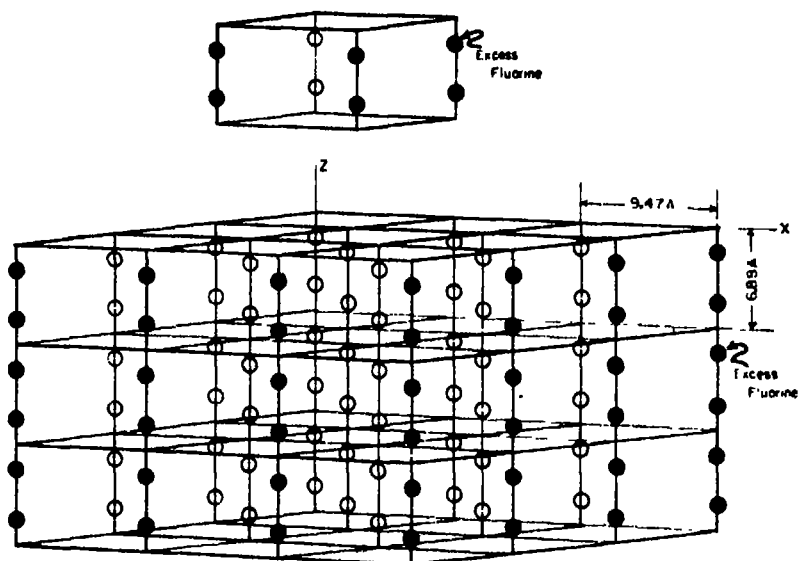
FIGURE 2

Excess fluorine in an apatite-type crystal due to lattice limitation.

are filled then part of the surface fluorine atoms would be in excess of the unit cell requirements as shown in figure 2. Francolite is always observed to be secondary in formation to collophane and as it forms with a decrease

TABLE 1

EFFECT OF FRAGMENT SIZE ON SURFACE AREA AND SOLUTION IN ACID OF SOME CARBONATE CONTAINING APATITE TYPE SUBSTANCES

SUBSTANCE	FINENESS SIEVE SIZE	SURFACE, M.²/G.	PARTIAL SOLUBILITY IN ACID, PER CENT OF TOTAL[a]		
			CO_2	CaO	P_2O_5
Steamed bone	100–200	66.2	18.6	6.5	6.5
	16– 30	66.2	15.5	5.9	5.4
	4– 8	63.7	..	...	...
Francolite	Below 6 micron	7.3	26.1	7.2	2.9
	100–200	3.2	10.5	6.6	3.9
	20– 35	1.6	6.5	3.7	3.5
Enamel	Through 325	6.8			
	200–325	3.7			
	120–200	3.2			
	60–120	2.1			

[a] Analyses by J. H. Caro.

in surface, excess fluorine present on the surfaces of primary collophane is exuded in limited amounts as secondary fluorite[14] in many phosphorites.

Other evidence for entrapped surfaces can be obtained from surface area measurements by nitrogen absorption (B.E.T.). These areas should depend markedly upon screen mesh size of material under test if entrapped surfaces are uncovered by breakage as indicated in figure 1. That this is true for enamel and francolite, but markedly less so for bone as required by the preferential solution of its carbonate in acid is shown by results in table 1. These surface areas were measured in the laboratory of Dr. V. R. Dietz of the National Bureau of Standards who, independently, had made the observations on enamel for Dr. R. C. Likens of the National Institutes of Health.

Attention is now turned to some detailed consideration of the surfaces. Bone, after removal of most of its organic matter, has a maximum surface of about 80–100 m.^{2}/g. (Reference 15 and extensive unpublished information of V. R. Dietz for bone ash) and contains about 7.5 $\times$ 10^{20} CO$_2$ groups/g.[7] The corresponding area per group, assuming a surface area of 100 m.^{2}/g., the larger value being taken in allowance for entrapped surfaces, is 13.3 A^2 which is about the value to be expected if the carbonate (and possibly citrate ions) forms a single surface layer containing cations and the maximum surface is realized.

Now consider the francolite sample analyzed by Gruner and McConnell[2] which contains 3.36% CO$_2$ and 1.08 times more fluorine than required for apatite. The excess surface fluorine is given by $2x + 1 = 0.08x^2$, where x is the number of primitive translations in a "crystallite" which is assumed to be equal in the x and y directions, figure 2. Thus x is about 25 corresponding to 234 A (25 $\times$ 9.35 A). The calculated "surface area"/g., made up chiefly of that entrapped, is about 62 m.^{2}/g. The "external surface" is surely less than 1 m.^{2}/g. A completely independent estimate of surface area is afforded by the carbonate content. For 3.36% CO$_2$ and an area of 13.3 A^2 per CO$_2$ group this is 61 m.^{2}/g. While the agreement with the other value is perfect the combination of the methods can only be trusted to about 25%, the greatest uncertainty being about the surface area per CO$_2$ group.

The nature of "tricalcium phosphate hydrate" having an apatite diffraction pattern, and serving as a prototype for bone, is now considered. This material can be formed by precipitating calcium phosphate in a system having a pH near that of plasma or by hydrolysis of CaHPO$_4$, i.e., under conditions where HPO$_4^{--}$ is a predominant phosphate ion in solution. The composition of this material determined by independent groups is Ca$_9$(PO$_4$)$_6$(H$_2$O)$_2$[4,7] or Ca$_9$(PO$_4$)$_6$(H$_2$O)$_{1.5}$,[16] the question of the exact water content of course being difficult to settle. A preparation made by slow hydrolysis of CaHPO$_4$ in a system more acid than pH 5.0 contained crystals measuring about 2 microns in length which were shown by electron micrographs to be well formed lath-like prisms with pyramidal faces. A ma-

terial of this type gradually lost 4.70% H_2O (calculated for $Ca_3(PO_4)_6(H_2O)_2$ 3.73% H_2O) between 100° and 800°. Above 800° the substance is essentially anhydrous and gives the diffraction pattern of the beta modification of $Ca_3(PO_4)_2$ (stable below 1115°C. and possibly a stable phase in solution) which verifies the molal ratio. Hydroxylapatite behaves in a markedly different way in retaining its full content of 1.79% H_2O upon heating at 1000°. It is dehydrated fully only above 1300°.

Is this substance really what it appears to be or is it hydroxylapatite as was suggested many years ago by Trömel[8] (also references 6 and 9) with surface material modifying the composition? The "formula" on this basis would be

$$Ca_{10}(PO_4)_6(OH)_2\text{—Surface } (PO_4)_{0.67}$$
$$\text{Surface } (H^+)_{2.00}$$
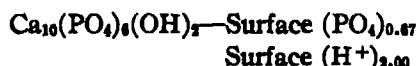
and would have to be approximated, on the basis of present evidence, over a pH range from about 4.6 to 7.3 in the precipitating system. The particular composition might vary somewhat and would be determined possibly by the surface and the pH. Surface areas of several materials made by precipitation varied from 26 to 64 m.^{2}/g. With surfaces, separated by perhaps ten primitive translations and entrapped in part, covered with HPO_4^{--} ions, behavior upon heating might parallel that observed; namely, gradual loss of water in a manner very different from that of hydroxylapatite. In the case of bone, HPO_4^{--} would have to be present on the surface together with carbonate and citrate. Experimental results at hand cannot safely resolve the question, although the authors, as well as Brasseur and Dallemagne, have previously considered the evidence adequate for "tricalcium phosphate hydrate." The observation that the poorly developed x-ray powder diffraction pattern is closely the same as that of hydroxyl apatite should surely be given full weight,[6] but it alone is not conclusive.

This surface chemistry of bone and related materials markedly affects composition and is a result or probable cause of the large surface area. It can be generalized. An insoluble calcium compound can form surface compounds with other ions having insoluble or poorly ionized calcium salts (e.g., citrate). Thus $CaCO_3$ would equally be expected to form surface phosphates and citrates in much the same way as calcium phosphates form surface carbonates. Such reactions which are important for phosphate fertility of calcareous soils have been observed in tracer experiments by Dr. Sterling Olson of this Bureau.

The phenomenon discussed here, which can be studied in many ways, has broad implications in the formation and properties of bone and teeth. An explanation is afforded for the unusual relationship of fluorite, collophane and francolite in phosphate rock. Interpretation can be given to

occurrence of uranium and other groups as surface material in phosphorites.[17]   An understanding is afforded for the preferential use of certain phosphate rocks, particularly those from North Africa having high carbonate contents, for direct application to some crops.[18]

Modification of composition by small particle size has also been found for hydroxylapatite by P. W. Arnold of Cambridge University (personal communication).   He observed that precipitates of hydroxylapatite contain $(OH)^-$ in excess of the formula requirements, such as to yield more than 2.0% water between 900° and 1400°C. (calculated 1.79% H_2O).

Summary.—The inorganic compounds of bone have a large surface on which carbonate, citrate, magnesium and sodium are located.   Carbonate is present on entrapped surfaces and not as constituent within the lattice of the mineral francolite, the typical "carbonate apatite," that also by lattice limitation, contains fluorine in excess of the fluorapatite requirement.   Enamel is similar to francolite in having entrapped surfaces.   The over-all composition, including lattice and surface, of bone is that of a neutral calcium phosphate while enamel is more basic, approximating hydroxylapatite.   The compound forming the lattice of bone remains to be established but it might well be hydroxylapatite with an excess of phosphate on the surface.

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LIMITATION OF ELECTRON DENSITY BY THE PATTERSON FUNCTION

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In several recent papers[1-3] it has been shown that the vector set can be solved for its fundamental set, and this implies, in a broad way, that a crystal structure can be solved from the intensities of its x-ray diffraction spectra. In particular, it implies that hitherto unsuspected relations exist between the Patterson function and the electron density function.

One such relation has been demonstrated: If an image of any polygon, whose n vertices ($n \geqslant 2$) have coordinates $x_1 y_1 z_1$, $x_2 y_2 z_2$, ..., $x_n y_n z_n$, can be found, one can construct the function

$$\Pi_n(xyz) = P(x_1 y_1 z_1) \times P(x_2 y_2 z_2) \times \ldots, P(x_n y_n z_n), \qquad (1)$$

where $P(x_1 y_1 z_1)$, etc., are Patterson functions. Then if there are no equal vectors between points in the fundamental set, there are no fortuitously coincident images in the vector set, and the following relation holds,[3] except for an exaggeration due to the selected image point:

$$\rho(xyz) = K \sqrt[n]{\Pi_n(xyz)}, \qquad (2)$$

where K is a constant defined by the chosen image.

For actual crystals, the electron density varies continuously with the coordinates, so that the Patterson synthesis contains a continuous overlapping of images. These arise not only from Patterson vectors radiating from a particular origin, but also from Patterson vectors reaching into a particular cell from the origins of the neighboring cells. The Patterson function for an actual crystal therefore contains extensively overlapped and coincident images. This feature always degrades the information obtainable from (1) in a specific way, namely by increasing the values of one or more P's and therefore increasing the value of Π. For an actual crystal, therefore, an inequality corresponding to (2) holds, namely

$$\rho(xyz) \leqslant K \sqrt[n]{\Pi_n(xyz)}. \qquad (3)$$

In spite of this unfortunate feature, the Π function is useful in the interpretation of Patterson maps. Only high ground can be occupied by atoms, and low ground can be regarded as unpermitted territory. In instances where the overlapping is minimized, the function is most useful. For example, a collection of heavy atoms embedded in a matrix of lighter ones can be readily located by the Π function. The reason for its power in such an instance is that the characteristics of the set of heavy atoms

approaches that of a set of pure points reasonably separated in empty space.

A particularly bad feature of the II function depends on the fact that a Patterson map usually has a more or less continuous non-zero background which arises from at least partial overlapping of the images of atoms. When a line image, for example, encounters a multiple peak at one end, it usually has this background at its other end, so that the product has some moderate value. Because of this, the II function often produces a ghost when one point of the polygon passes over a large Patterson peak, in spite of the fact that the polygon location is not an image position. Due to the presence of such ghosts, the II function usually has more peaks than the electron density function.

<table>
<tr><td>FIGURE 1
Centrosymmet-
rical fundamental
set.</td><td>FIGURE 2
Vector set of the fundamental
set of Fig. 1, showing weighting
of points (circles) and also
values of the M_2 function
(crosses).</td></tr>
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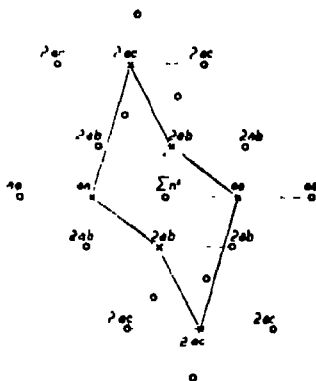
Obviously, the II function is not the only image-seeking function which can be devised to locate atoms. Any function which drops to zero when the weighting on at least one of the polygon vertices is zero can be expected to map the electron density with more or less faithfulness. A particular function which avoids the disadvantage of the II function mentioned in the last paragraph may be called an M function. To form the M_n function, one records at xyz the minimum value of the n values of the Patterson function which occur at the n vertices of the roving image polygon. It will be demonstrated that this function has the interesting property that

$$\rho(xyz) \leqslant K M_n(xyz), \tag{4}$$

using the same notation as for (2). Inequality (4) is more powerful than (3).

The general properties of the M function can be readily appreciated if it is applied to the case of a polygon image of n points which are related by symmetry in the crystal. For simplicity and clearness, consider $n = 2$, that is, a line image, and apply this image to a vector point-set. Let the point at the end of the origin line-image be a product ("interaction") between centrosymmetrically related points in the crystal. This is illustrated in figures 1 and 2. Figure 1 shows the points in a cell of the crystal and their weights; figure 2 shows the vector points arising from the points within the single cell shown in figure 1. The dotted lines of figure 2 show the line images, and the cross on each one shows the location of a peak of the M_2 function. The labeling indicates the weights of the peaks. Incidentally, note that all points of the vector set which are "interactions" between centrosymmetrical points of the crystal set have unit weight, while other "interaction" points have double weight. It will also be observed that the locations of the peaks of the M_2 function are the same as the points of the crystal set, and their magnitudes are the same except for a factor $2a$, which depends on the weight (corresponding to electron density) of point a, which was chosen for the first image point. An exception in weighting in the M function occurs for the point reproduced by the line image containing the origin; this has unit weight. The exception occurs only in the case of the point associated with the vector peak chosen for the line image to be used for the decomposition of the vector map.

The M function not only has the advantage mentioned above over the Π function, but also a related advantage of usually giving a more correct value when the image polygon comes to an image location for which one of the vertices lies on a multiple peak. As the Π function ranges over such an overlapped peak, it lays down an exaggerated peak, but the M function lays down a peak of normal magnitude provided that at least one vertex of the image polygon is not overlapped. Under such conditions, therefore (except for the neighborhood of. the atom a used as the initial image points) the M function has the important property

$$\rho(xyz) = \frac{1}{2\rho_a} M(xyz). \tag{5}$$

In the case of an actual crystal, the fundamental set becomes the electron density map, and the vector set becomes the Patterson map.[3] There is usually overlapping to some extent at all vertices of the image polygon in the Patterson map. But even when this occurs, it merely increases the value of M, and the inequality

$$\rho(xyz) \leqslant \frac{1}{2\rho_a} M(xyz) \tag{6}$$

is valid. Relation (6) implies that the Patterson function places an upper allowable limit of the electron density at every point in the cell. Since the electron density at the center of an atom is a characteristic of an atom, this relation will permit locating atoms in instances where the Patterson is not complicated by too much overlapping. In any case, it permits eliminating regions where a specific atom cannot be located, on the ground that the electron density at the atom center is greater than that of the M function in the region.

In crystals having symmetry other than an inversion center, a centro-symmetrical fraction of the cell is repeated by other symmetry elements to form the entire cell. When the image polygon is chosen so that it does not conform to the Patterson symmetry at the origin, it does not, in general, encounter symmetrical situations at equivalent locations in these fractions of the cell. Nevertheless, if the Patterson map contains no fortuitous overlaps, any image function synthesizes the true symmetry of the crystal. But if there are fortuitous overlaps, the image function may synthesize a degradation of the true symmetry. For example, consider a two-dimensional Patterson map of symmetry $p2mm$, and let a line image be chosen which, at the origin, makes an oblique angle with both symmetry lines. In reproducing symmetrically located peaks on the two sides of a symmetry line, the sloping image line uses two peaks on one side of the symmetry line and two peaks on the other side of the line, respectively. These two pairs of peaks are not symmetrically related unless the line image conforms to the symmetry of the Patterson map. Since, in general, each pair of peaks is involved in different overlaps, any line function reproduces the symmetry of the crystal as degraded by the overlap characteristic of the cell fraction. In such instances, the minimum value of either the Π or the M function at equivalent points in different cell fractions can be combined to give a better minimum value of Π or M, thus increasing the power of the particular inequality (3) or (6).

Since (6) is quantitative, it requires the Patterson synthesis upon which it is based to be quantitative. Any feature which reduces the accuracy of the Patterson synthesis therefore reduces the effectiveness of (6). A particularly bad effect has been noticed which is caused by the limited number of experimental F^2's available and the consequent termination of the Fourier series used for the synthesis of the Patterson map. This gives rise to false sets of undulations corresponding to optical diffraction fringes. A set of such undulations surrounds each strong Patterson peak, but the undulations are most noticeable surrounding the great origin peak. The ring-shaped trough and the following ring-shaped hill which immediately surround the origin are very objectionable and tend to distort

the value of the M function as the image polygon passes over them. It is recommended that before computing an M function from Patterson data these undulations be eliminated along the lines suggested by James.[4]

As described above, the M function was applied to polygon images composed of points due to symmetrical "interactions." It can also be applied to image polygons containing points due to non-symmetrical "interactions." The selected image is then a polygon whose n points have weights, say, $aa, ab, ac, \ldots$. To form the M function for this polygon, one first prepares n Patterson maps of the sampling of the Patterson function scaled, respectively, by factors $1/a, 1/b, 1/c, \ldots$, and lets one point of the image polygon rove over the corresponding map. When the polygon is located at xyz, the minimum value which occurs at the n points is then recorded at xyz, and the following relation holds

$$\rho(xyz) \leqslant M(xyz). \tag{7}$$

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SOME LINEAR OPERATORS IN THE THEORY OF PARTIAL DIFFERENTIAL EQUATIONS*

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1. Let D_2 be a finite domain in the x, y-plane bounded by an analytic curve C_2. Let D_1 be a proper subdomain of D_2 with analytic boundary curve C_1. Denote finally the difference domain bounded by C_1 and C_2 by D_0.

Consider the partial differential equation of elliptic type

$$\Delta u = q(P)u, \qquad \Delta = \frac{\partial^2}{\partial x^2} + \frac{\partial^2}{\partial y^2} \tag{1}$$

where $q(P)$ is a positive continuously differentiable function of the point $P = (x, y)$ in $D_2 + C_2$. We introduce the scalar products

$$E_i\{u, v\} = \int\int_{D_i} [\text{grad } u \cdot \text{grad } v + quv]d\tau, \qquad i = 0, 1, 2, \tag{2}$$

$$d\tau = \text{area element in } D_t$$

and the three Hilbert spaces Σ_t consisting of all solutions u of (1) with a finite norm $E_t(u) = E_t\{u, u\}$. Let finally $N_t(P, Q)$ and $G_t(P, Q)$ denote Neumann's and Green's functions with respect to the differential equation (1) and the domain D_t.

We define the kernels

$$K_t(P, Q) = N_t(P, Q) - G_t(P, Q), \ L_t(P, Q) = N_t(P, Q) + G_t(P, Q)$$
$$(3)$$

which play an important role in the theory of the spaces Σ_t [1, $a - d$; 2] and each of which is defined in the domain D_t. We may construct now discontinuous solutions of (1) which are defined in D_2, composed in a simple way of the kernels (3) and possess various interesting properties. We set

$$l(P, Q) = \begin{cases} L_1(P, Q) - L_2(P, Q) & \text{for } P, Q \ \epsilon \ D_1 \\ \quad\quad\quad - L_2(P, Q) & \text{for } P \ \epsilon \ D_1, Q \ \epsilon \ D_0 \text{ or } P \ \epsilon \ D_0, Q \ \epsilon \ D_1 \\ L_0(P, Q) - L_2(P, Q) & \text{for } P, Q \ \epsilon \ D_0. \end{cases}$$
$$(4)$$

Similarly, we define

$$k(P, Q) = \begin{cases} K_1(P, Q) - K_2(P, Q) & \text{for } P, Q \ \epsilon \ D_1 \\ \quad\quad\quad - K_2(P, Q) & \text{for } P \ \epsilon \ D_1, Q \ \epsilon \ D_0 \text{ or } P \ \epsilon \ D_0, Q \ \epsilon \ D_1 \\ K_0(P, Q) - K_2(P, Q) & \text{for } P, Q \ \epsilon \ D_0 \end{cases}$$
$$(5)$$

Using the characteristic properties of the various Green's and Neumann's functions, the following identity can be established:

$$E_2\{l(P, Q), l(P, R)\} = k(Q, R), \quad Q, R \ \epsilon \ D_2 \text{ but not on } C_1 \quad (6)$$

Here the convention will be used that if kernels with various argument points are multiplied the operation shall always be understood with respect to the common argument letter. Similarly, we can show

$$E_2\{l(P, Q), k(P, R)\} = l(Q, R), \quad Q, R \ \epsilon \ D_2 \text{ but not on } C_1, \quad (6')$$

and

$$E_2\{k(P, Q), k(P, R)\} = k(Q, R), \quad Q, R \ \epsilon \ D_2 \text{ but not on } C_1 \ (6'')$$

2. Let Σ denote the Hilbert space of all functions in D_2 which represent in D_0 an element of Σ_0 and in D_1 an element of Σ_1. The metric in Σ will be based on the scalar product $E_2\{u, v\}$.

We define in Σ the linear operators [cf. references 1d and 3]

$$T_u(P) = E_2\{l(P, Q), u(Q)\}, \quad S_u(P) = E_2\{K_2(P, Q), u(Q)\}, \ (7)$$

which transform the space Σ into itself. They satisfy the following relations:

$$E_2\{u, v\} = E_2\{T_u, T_v\} + E_2\{S_u, S_v\} \tag{8}$$
$$E_2\{T_u, S_v\} = 0. \tag{8'}$$

These results suggest the introduction of the two linear operators in Σ:

$$\mathfrak{S}_u{}^+ = T_u + S_u \qquad \mathfrak{S}_u{}^- = T_u - S_u \tag{9}$$

which have the property of preserving the metric

$$E_2\{\mathfrak{S}_u{}^+, \mathfrak{S}_v{}^+\} = E_2\{u, v\}, \qquad E_2\{\mathfrak{S}_u{}^-, \mathfrak{S}_v{}^-\} = E_2\{u, v\}. \tag{10}$$

The operators $\mathfrak{S}_u{}^+$ and $\mathfrak{S}_u{}^-$ have further the following involutory character:

If $v = \mathfrak{S}_u{}^+$, then $u = \mathfrak{S}_v{}^+$ and if $w = \mathfrak{S}_u{}^-$ then $u = \mathfrak{S}_w{}^-$. This result shows that $\mathfrak{S}^+$ and $\mathfrak{S}^-$ are one-one mappings of Σ onto itself.

Each function $u \, \epsilon \, \Sigma$ can be decomposed in a unique way into

$$u(P) = a(P) + b(P) \tag{11}$$

where $b(P) \, \epsilon \, \Sigma_2$ and $a(P)$ is orthogonal to Σ_2. The operators T and S perform this orthogonal decomposition of Σ.

The operators $\mathfrak{S}^+$, $\mathfrak{S}^-$ can easily be interpreted as follows: Given an element $u \, \epsilon \, \Sigma$, which is continuously differentiable in the closures of D_0 and D_1, $\mathfrak{S}_u{}^+$ will be that function of Σ which has on C_2 the same boundary values as u, which has along C_1 the same discontinuity a s u but whose normal derivative has the discontinuity of opposite sign on C_1 than has $\dfrac{\partial u}{\partial \nu}$.

Similarly $\mathfrak{S}_u{}^-$ has on C_2 the same normal derivative as u, its normal derivative has the same jump through C_1 as that of u, but its discontinuity across C_1 is at each point minus the jump of u over that line.

3. Let $u \, \epsilon \, \Sigma$ be identically zero in D_0. In this case $E_2(u) = E_1(u)$ and we derive from (8):

$$E_1(u) \geqslant E_1(T_u). \tag{12}$$

This inequality holds for every function $u \, \epsilon \, \Sigma_1$. We derive from it, in particular, that the eigenvalues of the integro-differential equation

$$u_\rho(P) = \lambda_\rho E_1\{l(P, Q), u_\rho(Q)\}, \qquad Q \, \epsilon \, D_1 \tag{13}$$

are all greater or equal to 1 in absolute value. A finer study of the conditions for the equality sign shows that always

$$|\lambda_\rho| > 1 \tag{14}$$

must hold. This result plays an important role in the theory of the kernels [3].

4. Let us define the linear operator in Σ_1

$$s_u{}^+(P) = E_1\{l(P, Q) + K_1(P, Q), u(Q)\}. \tag{15}$$

From the continuity properties of the kernels l and K_1 one can easily show that this operator is completely continuous and, hence, treat the eigenvalue problem

$$u_\rho(P) = \kappa_\rho s_{u_\rho}{}^+(P), \qquad P \epsilon D_1, \tag{16}$$

by the classical methods of Hilbert space theory. We obtain a discrete spectrum of eigenvalues κ_ρ and corresponding eigenfunctions $u_\rho(P)$ which we may assume to form a complete orthonormal set in Σ_1. By the reasoning of the last section, one can easily prove

$$|\kappa_\rho| > 1. \tag{16'}$$

We have in D_1 the Fourier developments

$$K_1(P, Q) = \sum_{\rho=1}^{\infty} u_\rho(P)u_\rho(Q), \qquad l(P, Q) + K_1(P, Q) = \sum_{\rho=1}^{\infty} \frac{1}{\kappa_\rho} u_\rho(P)u_\rho(Q). \tag{17}$$

Using the definitions (3), we easily derive from (17):

$$G_1(P, Q) - G_1(P, Q) = \frac{1}{2} \sum_{\rho=1}^{\infty} \left(1 - \frac{1}{\kappa_\rho}\right) u_\rho(P)u_\rho(Q). \tag{18}$$

Consider the $\mathfrak{S}^+$-transforms of the eigenfunctions $u_\rho(P) \epsilon \Sigma_1$ which are extended into functions of the class Σ by defining them to be identically zero in D_0. Let

$$v_\rho(P) = \mathfrak{S}_{u_\rho}{}^+(P) = -2E_1\{G_1(P, Q), u_\rho(Q)\}, \qquad P \epsilon D_0. \tag{19}$$

The $\{v_\rho(P)\}$ form an orthogonal set of functions in Σ_0; they satisfy

$$v_\rho(P) = -\kappa_\rho E_0\{l(P, Q) + K_1(P, Q), v_\rho(Q)\} \tag{20}$$

and thus play a very similar role in Σ_0 as did the u_ρ in Σ_1. We calculate

$$E_0\{v_\rho, v_\sigma\} = \left(1 - \frac{1}{\kappa_\rho{}^2}\right) \delta_{\rho\sigma}. \tag{21}$$

All $v_\rho(P)$ vanish on C_1; they form a complete set of functions in the subspace of Σ_0 which consists of all elements vanishing on C_1. The function $G_1(P, Q) - G_0(P, Q)$ belongs to this subspace and can, therefore, be developed into a Fourier series in terms of the $v_\rho(P)$:

$$G_1(P, Q) - G_0(P, Q) = \frac{1}{2} \sum_{\rho=1}^{\infty} \frac{\kappa_\rho}{\kappa_\rho - 1} v_\rho(P)v_\rho(Q). \tag{22}$$

Since the $v_\rho(P)$ can be calculated from the $u_\rho(P)$ by elementary integra-

tion if G_1 is known, we see that the $u_\rho(P)$ permit a representation of the Green's functions of the complementary domains D_0 and D_1.

Using instead of s^+ the linear operator

$$s_u^-(P) = E_1\{l(P, Q) - K_1(P, Q), u(Q)\} \tag{23}$$

we can develop an analogous theory for Neumann's functions.

5. The function $w_\rho(P) \in \Sigma$ which has the value $\dfrac{1}{\kappa_\rho} u_\rho(P)$ in D_1 and $v_\rho(P)$

in D_0 is the $\mathfrak{S}^+$-transform of the function $u_\rho \in \Sigma$ which has been extended from Σ_1 by defining it identically zero in D_0. Using the known discontinuities of the function u across C_1 we derive

$$v_\rho(P) = -\left(1 - \frac{1}{\kappa_\rho}\right) u_\rho(P), \qquad\qquad P \in C_1 \tag{24}$$

$$\frac{\partial v_\rho(P)}{\partial \nu} = \left(1 + \frac{1}{\kappa_\rho}\right) \frac{\partial u_\rho(P)}{\partial \nu}, \qquad\qquad P \in C_1 \tag{24'}$$

Thus, the boundary values and the values of the normal derivatives of the functions $v_\rho \in \Sigma_0$ and $u_\rho \in \Sigma_1$ are proportional along the common boundary C_1 of their domains of definition.

The function $\omega_\rho(P) \in \Sigma$ which is defined as $\left(\dfrac{1}{\kappa_\rho} - 1\right) u_\rho(P)$ in D_1 and as $v_\rho(P)$ in D_0 has the following property: It is continuous in D_2, vanishes on the boundary C_2 of D_2 and satisfies along the curve C_1 the discontinuity condition

$$\frac{\partial \omega_\rho}{\partial \nu^+} = -\frac{\kappa_\rho + 1}{\kappa_\rho - 1} \frac{\partial \omega_\rho}{\partial \nu^-}. \tag{25}$$

Thus, the eigenvalue κ_ρ can also be characterized by an eigenvalue problem for discontinuous solutions of (1) of the Stekloff type [4].

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GENERALIZED GROUP ALGEBRAS

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The purpose of this note is to describe a natural banach algebra associated with any topological group; the theory of this algebra and its subalgebras subsumes much of the standard L^1 theory, and is independent of the existence of a measure. Proofs will appear elsewhere.[1]

Let G be an arbitrary topological group, not necessarily locally compact, and let K be the complex field. Let C be the banach space of all bounded *uniformly* continuous functions on G to K, with norm $\|f\| = \sup_{x \in G} |f(x)|$.

Let $B(C)$ be the banach algebra of all bounded linear operators on C. For $b \in G$, let U_b be the left translation operator which sends a function f into $_bf$ whose value at x is $f(bx)$, and let U^b be the corresponding right translation. By our choice of C, U_b and U^b are in $B(C)$ and have norm 1; the mapping $x \to U_x$ $(x \to U^x)$ is an anti-isomorphism (isomorphism) of G in $B(C)$, not in general continuous.

DEFINITION: *Let $\mathfrak{B}$ be the set of all T in $B(C)$ which commute with all the operators U_b.*

$\mathfrak{B}$ is a closed subalgebra of $B(C)$; it is the centralizer in $B(C)$ of the subalgebra generated by the left representation of G. Since U^x and U_y commute, $\mathfrak{B}$ contains the right representation of G in $B(C)$, and we may therefore regard G as embedded in $\mathfrak{B}$. Consider $\hat{G}$, the set of all bounded continuous homomorphisms of G into K°, the multiplicative group of K. Clearly, $\hat{G}$ is a subset of the unit sphere in C.

THEOREM 1. *A function f in C is a character or a multiple of a character if and only if f is simultaneously an eigenfunction for every operator in $\mathfrak{B}$.*

Thus, $\mathfrak{B}$ determines $\hat{G}$ and hence G, when G is such that the duality theorem holds. Regarding G as embedded in $\mathfrak{B}$, it has two topologies, the group topology carried over by the embedding, and the relative norm topology which it acquires as a subset of $\mathfrak{B}$; the latter is usually discrete.

THEOREM 2. *Any character of G has an extension to a ring homomorphism of $\mathfrak{B}$ into K, continuous in the norm topology.*

If $h \in \hat{G}$, then the extension H is defined by $H(T) = \lambda$, where λ is the eigenvalue of h under T. The kernel of H is simply the set of all T in $\mathfrak{B}$ with $T(h) = 0$, the origin of C.

COROLLARY: *$\hat{G}$ has a natural one-to-one embedding in $Hom(\mathfrak{B}:K)$ the set of all continuous ring homomorphisms of $\mathfrak{B}$ onto K.*

Clearly, any homomorphism H of $\mathfrak{B}$ onto K has a contraction to G which is a group homomorphism of G into K°. (This remark, together with the preceding theorem, essentially contains the results obtained by Kakutani

and Kodaira.[2]) However, this homomorphism is not necessarily in $\check{G}$ since it need not be continuous in the group topology of G. Under certain conditions, this continuity can be forced. For example, this is the case if there is an operator $T_0 \, \epsilon \, \mathfrak{B}$ such that $H(T_0) = 1$ while the map $x \to U^x T_0$ is continuous. This is the mechanism underlying the usual L^1 theory.

Since $\mathfrak{B}$ is large, the study of its subalgebras is of importance. Let $\mathfrak{A}_0$ be the closed subalgebra generated by the image of G in its natural embedding. We call a subalgebra $\mathfrak{A}$ total if $T(f) = 0$ for every $T \, \epsilon \, \mathfrak{A}$ implies that $f = 0$. It is convenient to single out the class of linear functionals L on $\mathfrak{B}^+$ having the form $L(T) = T(\varphi)(e)$ where $\varphi \, \epsilon \, C$ and e is the neutral element of G.[3] We term such regular functionals; they form a subspace of the dual of $\mathfrak{B}^+$, usually not closed. The homomorphisms which arise as extensions of those in G are regular. Given a subalgebra $\mathfrak{A} \subset \mathfrak{B}$, $\hat{G}$ has a natural one-to-one embedding in $\mathrm{Hom}(\mathfrak{A}:K)$ which in certain cases is onto.

THEOREM 3. *Let $\mathfrak{A}$ be a closed total subalgebra of $\mathfrak{B}$ such that (1) $\mathfrak{A}_0\mathfrak{A} \subset \mathfrak{A}$ and $\mathfrak{A}\mathfrak{A}_0 \subset \mathfrak{A}$, (2) the map $x \to U^x T$ is continuous for each $T \, \epsilon \, \mathfrak{A}$, (3) if H is any homomorphism of $\mathfrak{A}$ onto K, then $H(U^b T) = H(T U^b)$ for all $b \, \epsilon \, G$ and $T \, \epsilon \, \mathfrak{A}$, (4) every member of $\mathrm{Hom}(\mathfrak{A}:K)$ is the limit of regular functionals. Then, every homomorphism of $\mathfrak{A}$ onto K arises as the unique extension of a member of $\hat{G}$.*

To show the connection of the preceding with the usual theory, let $\mathfrak{L}$ be the dual space of C. The correspondence $T \to F$, where $F(f) = T(f)(e)$ may be shown to be a norm preserving isomorphism of the banach spaces $\mathfrak{B}$ and $\mathfrak{L}$. Since $\mathfrak{B}$ has the additional structure of an algebra, $\mathfrak{L}$ can be given the same structure. Denoting the resulting multiplication for functionals by $*$, $F_1{}^* F_2$ turns out to be the customary convolution of the functionals F_1 and F_2, in the sense of Radon measures.[4] The operator U^b in $\mathfrak{B}$ corresponds to the point functional θ_b where $\theta_b(f) = f(b)$. The theory of the Fourier transform assumes the following simple form: given $F \, \epsilon \, \mathfrak{L}$, let $\hat{F}$ be its restriction to the subset $\hat{G} \subset C$. The importance of discussing the span of $\hat{G}$ in C is evident. For the usual L^1 theory, choose G to be locally compact abelian, and let $\mathfrak{A}$ be the set of all operators of the form $g(x) = \int f(xy)\varphi(y) \, dy$ where $\varphi \, \epsilon \, L^1(G)$ and $f \, \epsilon \, C$. The hypotheses of Theorem 3 are easily verified.

The norm topology on C is not the only usable one; in particular, that of uniform convergence on compact sets deserves special consideration. The results for this case are similar, and will appear elsewhere.

[1] There seems to be some relation between the present approach and that in a recent abstract of Hewitt and Zuckerman, *Bull. Am. Math. Soc.*, **55**, 1057 (1949). See also I. E. Segal, *Ann. Math.*, **51**, 293–298 (1950). The two-sided regular representation of a unimodular locally compact group.

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³ B^+ is the underlying banach space of the algebra B.

⁴ Weil, *L'integration dans les groupes topologiques*, Actualitiés Sci. et Indust., Paris, 1940.

LIMIT CYCLES OF SYSTEMS OF THE SECOND ORDER

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The present paper deals with a number of questions about the limit cycles of systems

$$\dot{x} = P(x, y), \qquad \dot{y} = Q(x, y). \tag{1}$$

The importance in non-linear mechanics of these limit cycles, or isolated periodic solutions, is well known. It has led to a variety of methods for finding these solutions which yield some information in noteworthy special cases. In Princeton lectures in the fall of 1949, Lefschetz introduced and applied to certain questions the following method: a rotation by a fixed angle α being applied to the vector field (X, Y) in the phase plane of the system (1) gave rise to a new vector field $(X \cos \alpha - Y \sin \alpha, X \sin \alpha + Y \cos \alpha)$, whose trajectories were then compared to those of the original system. A method essentially like that of Lefschetz is utilized in this note, and has made it possible to answer systematically many questions concerning limit cycles of second order systems.

Consider then a family of systems

$$\dot{x} = P(x, y, \alpha), \qquad \dot{y} = Q(x, y, \alpha). \tag{2}$$

We shall say that these equations form a complete family, and that the associated vector fields in the (x, y) plane form a complete family of rotated vector fields in the plane, if the following conditions are satisfied:

(a) P and Q satisfy Lipschitz conditions with respect to x and y, and are differentiable functions of α.

(b)   The zeros of $P^2 + Q^2$ remain fixed in the (x, y) plane as α varies.

(c)   The condition

$$P \frac{\partial Q}{\partial \alpha} - Q \frac{\partial P}{\partial \alpha} > 0$$

is satisfied at all points where $P_2 + Q^2 > 0$.

(d) P and Q are periodic functions of α with minimum period 2π, and furthermore

$$P(x, y, \alpha + \pi) = -P(x, y, \alpha),$$
$$Q(x, y, \alpha + \pi) = -Q(x, y, \alpha).$$

As α varies, one may picture the field vectors as turning, at a positive rate, all in the same sense, and in such a way that, in the interval $0 \leq \alpha < \pi$, all have turned through the angle π. Thus the original field, but with the vectors reversed, appears again. An example is the set of uniform rotations of the field vectors by an angle α. The critical points remain fixed, according to condition (*b*). We shall study the phase portraits of these fields, and will show that they are closely related.

We note that the index (in the sense of Poincaré) of any closed curve in the plane, with respect to any field of a complete family, is independent of α. It may also be verified that if a change of the dependent variables of the form $x_1 = x_1(x, y)$, $y_1 = y_1(x, y)$, with functional determinant everywhere different from zero, is made, then the differential equations in x_1 and y_1 also constitute a complete family.

The following theorems give fundamental properties of complete families as defined above:

THEOREM I. *Limit cycles corresponding to different values of α do not intersect.*

THEOREM II. *Limit cycles which are completely stable or completely unstable expand or contract monotonely as α varies in a fixed sense. Semistable cycles either split into two diverging cycles or disappear.*

THEOREM III. *Each elementary critical point of positive determinant Δ either generates or absorbs exactly one cycle in the range $0 \leq \alpha < \pi$.*

We sketch a proof of Theorem III for the case in which the rotations are uniform over the plane. Let

$$\dot{x} = X \cos \alpha - Y \sin \alpha,$$
$$\dot{y} = X \sin \alpha + Y \cos \alpha,$$

be the equations of the family, where

$$X = ax + by + X_2,$$
$$Y = cx + dy + Y_2,$$

and X_2 and Y_2 contain only terms of the second and higher orders in x and y. The characteristic roots of the linear equations of first approximation near the origin are given by

$$\lambda^2 - [(a + d) \cos \alpha + (b - c) \sin \alpha] \lambda + ad - bc = 0.$$

Writing

$$\Delta = ad - bc,$$
$$\Gamma^2 = (a + d)^2 + (b - c)^2,$$

and

$$\tan \delta = \frac{a + d}{c - b},$$

we can write the characteristic roots in the form

$$\lambda = +\frac{\Gamma}{2}\sin{(\alpha - \delta)} \pm \frac{\sqrt{\Gamma^2 \sin^2{(\alpha - \delta)} - 4\Delta}}{2}.$$

If $\Delta > 0$, corresponding to an index of $+1$, these roots assume both real and complex values, since $4\Delta \leq \Gamma^2$. For $\alpha = \delta$, the roots are purely imaginary, and the origin is a center. For nearby values of α, the origin is a focus, or spiral point, with the stability of the spirals changing sense when $\alpha = \delta$. According to Theorem I, the generation of such a cycle can take place only once in a half revolution of the field.

With the aid of these theorems, it can be shown that regions of certain types in the phase plane are swept out by the cycles of a given complete family. Examples of such regions are: the interior of a given cycle containing a single critical point, which is elementary, or an annulus free of critical points which is bounded by two cycles of the family not necessarily belonging to the same value of α. In some cases the whole plane is covered. These results, together with Theorem I, lead to criteria for the non-existence in such regions of limit cycles of other equations of the given complete family.

The theorems quoted above also suggest the following method of locating limit cycles. Embed the given equation in a complete family of rotations and calculate the values of δ for the elementary critical points of index $+1$. The expansion of a cycle generated at one of these points may be calculated as follows: When the cycle is very small, the linear terms in the expansions of Theorem III predominate in the right-hand sides of the equations and it is possible to use Poincaré's method of generating cycles to determine the size. This method picks out the closed solution of an equation with small non-linear terms from among the closed solutions of the corresponding linear equation. The rate of expansion may subsequently be found with the following formula for $\dfrac{dn}{d\alpha}$ the rate of expansion of the cycle along its normal: Let T be the period and h the exponent of the cycle. Let $v(t)$ denote the "velocity" $(P^2 + Q^2)^{1/2}$. Then,

$$\frac{dn(t)}{d\alpha} = \frac{\oint \exp\left[-\int_t^{t+\tau}(P_x + Q_y)d\tau'\right]\left\{P\dfrac{\partial Q}{\partial \alpha} - Q\dfrac{\partial P}{\partial \alpha}\right\}_{t+\tau} d\tau}{v(t)\,[1 - \exp(-hT)]}.$$

The cycle may disappear in one of the following ways: (a) by coalescing with an exterior or interior cycle of opposite stability (in this case, reverse the variation of α and follow the other cycle); (b) by tending to a saddle point or other critical point; (c) by tending to infinity in one or more directions.

If the cycle in question fails to persist until the value of α correspond-ing to the given equations is reached, then Theorem I asserts that no part of any cycle of this system lies in the region swept out during the expansion. For any value of α the cycle may become a band of positive width of closed cycles. When α has run through an interval of length π, each cycle must either have disappeared or else have taken the place of another cycle of the original field. Thus the method will always find those limit cycles which enclose one critical point with $\Delta > 0$, and it provides a criterion of non-existence, in case there are no such cycles.

In more complicated situations where the cycle might surround several critical points one must establish the existence of a cycle of the family for some value of α. This may be accomplished by finding a closed curve with-out contact with the given field (a Bendixson curve), and then choosing the complete family in such a way that this curve becomes a limit cycle for a particular value of α.

THEOREM IV. *All cycles of a given field containing the same critical points as a given Bendixson curve can be constructed by rotation operations of a suit-able complete family.*

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ON THE ADDITION AND MULTIPLICATION THEOREMS FOR SPECIAL FUNCTIONS*

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Some years ago I called attention to the unification in the theory of special functions achieved through systematic exploitation of properties of the solutions of the F-equation[1]

$$\frac{\partial F(z, \alpha)}{\partial z} = F(z, \alpha + 1). \tag{1}$$

One virtue of the method is that many formal relationships are discovered and demonstrated in an automatic and trivial fashion, relationships which, if previously known at all, were originally derived at greater length and with more elaborate analytical tools. In particular, if $F(z + y, \alpha)$ be an analytic function of y, we have by Taylor's theorem the addition formula

$$F(z + y, \alpha) = \sum_{n=0}^{\infty} \frac{y^n}{n!} F(z, \alpha + n), \tag{2}$$

which contains as special cases most of the addition formulae and many of the generating expansions for familiar special functions.[2] In this note I wish to point out that this same expansion may be written as a multiplication theorem. First we put $y - z$ for y in (2):

$$F(y, \alpha) = \sum_{n=0}^{\infty} \frac{(y - z)^n}{n!} F(z, \alpha + n). \tag{3}$$

If $y = kz$ then (3) becomes the multiplication theorem

$$F(kz, \alpha) = \sum_{n=0}^{\infty} \frac{(k - 1)^n z^n}{n!} F(z, \alpha + n), \tag{4}$$

which is the desired result.

If, in the general multiplication theorem (4), we put

$$F(z, \alpha) = e^{i\alpha\pi} z^{-\alpha/2} J_\alpha (2\sqrt{z}), \tag{5}$$

where $J_\alpha(x)$ is the Bessel function of the first kind, after some reductions we readily obtain the known result[3]

$$J_\alpha(kz) = k^\alpha \sum_{n=0}^{\infty} \frac{(1 - k^2)^n}{n!} \left(\frac{z}{2}\right)^n J_{\alpha+n}(z). \tag{6}$$

If J_α be replaced by Y_α, (6) remains valid. If instead we put

$$F(z, \alpha) = \Gamma(\alpha + 1) (-z)^{-\alpha-1-b} e^{-\frac{1}{z}} L_\alpha^{(b)} (1/z) \tag{7}$$

where $L_\alpha^{(b)}(z)$ is the generalized Laguerre function, by substitution in (5) follows the first multiplication theorem of Erdélyi:[4]

$$k^{\alpha+1+b} e^{-kz} L_\alpha^{(b)} (kz) = \sum_{n=0}^{\infty} \binom{\alpha+n}{n}\left(1 - \frac{1}{k}\right)^n e^{-z} L_{\alpha+n}^{(b)} (z), \tag{8}$$

while if we put

$$F(z, \alpha) = e^{i\alpha\pi} e^{-z} L_b^{(\alpha)} (z), \tag{9}$$

we similarly obtain the second multiplication theorem of Erdélyi:[5]

$$e^{-kz} L_b^{(\alpha)} (kz) = \sum_{n=0}^{\infty} \frac{(1 - k)^n z^n}{n!} e^{-z} L_b^{(\alpha+n)} (z). \tag{10}$$

Of the at least eleven multiplication theorems which hold for the hypergeometric functions, it may be of interest to write down two of the simplest, those resulting from the respective choices

$$F(z, \alpha) = e^{i\alpha\pi} \Gamma(\alpha) z^{-\alpha} F\left(\alpha, c; b; \frac{1}{z}\right), \tag{11}$$

$$F(s, \alpha) = \frac{\Gamma(\alpha - b)\,\Gamma(\alpha - c)}{\Gamma(\alpha)}(1 - s)^{b+c-\alpha}F(b, c;\ \alpha;\ s), \quad (12)$$

namely,

$$F(\alpha, b; c; ks) = k^{-\alpha}\sum_{n=0}^{\infty}\binom{\alpha+n-1}{n}\left(1 - \frac{1}{k}\right)^{n}F(\alpha + n, b; c; s).$$

$$(13)$$

$$F(a, b; c; ks) = \left(\frac{1-s}{1-ks}\right)^{a+b-c}\sum_{n=0}^{\infty}\left(\frac{k-1}{1-\frac{1}{s}}\right)^{n}\binom{1-c+a}{n}\binom{1-c+b}{n}\binom{n}{1-c}$$

$$F(a, b; c + n; s). \quad (14)$$

Finally, we may put

$$F(s, \alpha) = e^{i\alpha\pi}\Gamma(\alpha)\zeta(\alpha, s), \quad (15)$$

where $\zeta(\alpha, s)$ is the generalized Euler-Riemann zeta function, thus obtaining the expansion

$$\zeta(\alpha, ks) = \sum_{n=0}^{\infty}\binom{\alpha+n-1}{n}(1 - k)^{n}s^{n}\,\zeta(\alpha + n, s), \quad (16)$$

a result which when $s = 1$ yields a formula for $\zeta(\alpha, k)$ as a series of simple zeta functions.

We conclude with three remarks. The first is that since all the results of this note have been derived by simple substitutions in (2), without use of any limit operation, and since (2) is valid whenever the series on the right converges, it follows that all our results are valid subject only to convergence of the series involved. The second is that since k and s occur symmetrically on the left side of (4), by interchanging them upon the right side and equating the results we obtain the reciprocal theorem

$$\sum_{n=0}^{\infty}\frac{(k - 1)^{n}s^{n}}{n!}F(s, a + n) = \sum_{n=0}^{\infty}\frac{(s - 1)^{n}k^{n}}{n!}F(k, \alpha + n), \quad (17)$$

subject to the convergence of both series. The third is that by putting $k = 0$ the formula (4) enables us to find

$$\phi(\alpha) = F(0, \alpha) = \sum_{n=0}^{\infty}\frac{(-)^{n}s^{n}}{n!}F(s, \alpha + n), \quad (18)$$

a result which is interesting both because the left-hand side is independent of s and because the function $\phi(\alpha)$ occurs explicitly in many of the theorems concerning solutions of the F-equation.

[1] Truesdell, C., *An Essay Toward a Unified Theory of Special Functions,* Princeton, 1948.

[2] Op. cit., ¶14.

[3] Watson, J. N., *A Treatise on the Theory of Bessel Functions,* Cambridge, 1922. See ¶5.22.

[4] Erdélyi, A., "Funktionalrelationen mit konfluenten hypergeometrischen Funktionen," I, *Math. Z.,* 42, 125–143 (1936–1937), eq. (5,3).

[5] Op. cit., eq. (6,2). In this note we employ the definition of the generalized Laguerre function given by E. Pinney, "Laguerre Functions in the Mathematical Foundations of the Electromagnetic Theory of the Paraboloidal Reflector," *J. Math. Phys.,* 25, 49–79 (1946), so that the lower index is not restricted to integer values. Thus our results (8) and (10) are fully equivalent to Erdélyi's formulae (5,1) and (6,1), which are expressed in terms of the $M_{k, m}$ functions of Whittaker. Generalizations of Erdélyi's formulae (5,4) and (6,3) may be obtained either by applying Kummer's first transformation to our expansions (8) and (10) or by inserting successively the two solutions $s^{-a}L_b^{(-a)}(s)/\Gamma(b - a + 1)$ and $\Gamma(a - b) (-s)^{-a}L_{-a}^{(b)}\left(\dfrac{1}{s}\right)$ into the general multiplication theorem (4).